UNIVERSIDAD COMPLUTENSE DE MADRID
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Departamento de Farmacología, Farmacognosia y Botánica

TESIS DOCTORAL

El ciclo del nitrógeno en la región subantártica chilena y la Antártida marítima. Principales factores ambientales que intervienen en su regulación

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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MEMORIA DE TESIS DOCTORAL PRESENTADA POR:

ALBERTO BENAVENT GONZÁLEZ

Dirigida por los doctores:

Leopoldo García Sancho, Fernando Tomás Maestre Gil y

Manuel Delgado Baquerizo

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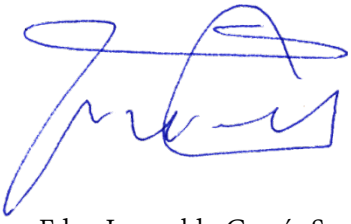
Don Leopoldo García Sancho, Doctor en Ciencias Biológicas y Catedrático de Farmacia en la Universidad Complutense de Madrid, Don Fernando Tomás Maestre Gil, Doctor en Biología y Catedrático de Ecología en la Universidad Rey Juan Carlos, y Don Manuel Delgado Baquerizo, Doctor en Ciencias Ambientales e investigador postdoctoral en la Universidad de Colorado Boulder informan que:

La memoria titulada: "El ciclo del nitrógeno en la región subantártica chilena y la Antártida marítima. Principales factores ambientales que intervienen en su regulación.", que presenta Alberto Benavent González, Licenciado en Ciencias Ambientales, para optar al grado de Doctor, ha sido realizada en el Departamento de Farmacología, Farmacognosia y Botánica de la Facultad de Farmacia de la Universidad Complutense de Madrid bajo su dirección, reuniendo todas las condiciones exigidas a los trabajos de tesis doctoral.

Madrid, 16 de julio de 2018



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Diario del viaje de un naturalista alrededor del mundo
Charles Darwin

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Molina, J.A., Lumbreras, A., **Benavent-González, A.**, Rozzi, R., Sancho, L.G. 2016. Plant communities as bioclimate indicators on Isla Navarino, one of the southernmost forested areas of the world. Gayana Botanica 73, 931-401.

Benavent-González, A., Delgado-Baquerizo, M., Hamonts, K., Molina, J.A., Singh, B.K., Maestre, F.T., & Sacho, L.G. 2018. Plant community attributes predict elevational changes in microbial diversity, abundance and co-occurrence networks in a Sub-Antarctic environment. Journal of Ecology. En revisión.

Benavent-González, A., Delgado-Baquerizo, M., Fernández-Brun, L., Singh, B.K., Maestre, F.T., & Sacho, L.G. 2018. Identity of plant, lichen and moss species connects with microbial abundance and soil functioning in Maritime Antarctica. Plant and Soil, DOI: 10.1007/s11104-018-3721-7.

Benavent-González, A., Raggio, J. Villagra, J. Blanquer, J.M., Pintado, A., Rozzi, R., Green, T.G.A., & Sancho, L.G. 2018. High nitrogen contribution by *Gunnera magellanica* and nitrogen transfer by mycorrhizae drive an extraordinarily fast primary succession in Sub-Antarctic Chile

Benavent-González, A., Green, T.G.A., Maestre, F.T., & Sancho, L.G. 2018. Elevational variation in morphology, nitrogen fixation and photosynthetic traits of *Gunnera magellanica* Lam. in a sub-Antarctic ecosystem.

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RESUMEN GENERAL

Introducción: La presente tesis doctoral, titulada "**El ciclo del nitrógeno en la región subantártica chilena y la Antártida marítima. Principales factores ambientales que intervienen en su regulación.**", estudia diferentes aspectos relacionados con la funcionalidad de los ecosistemas antárticos y subantárticos. La zona de estudio comprende la región de Tierra del Fuego, ubicada en el extremo más meridional del continente americano, y el archipiélago de las Islas Shetland del Sur, incluido en la región de la Antártida marítima y próximo a la Península Antártica. Estas regiones incluyen semejanzas, pero también importantes diferencias. Por ejemplo, a pesar de que distan apenas 900 km, ambas regiones contrastan por sus características ecológicas. Así, la región de Tierra del Fuego es la región que alberga las masas forestales más australes del planeta, y por lo tanto más próximas al continente antártico, que se caracteriza por la casi total ausencia de plantas vasculares (a excepción de *Deschampsia antarctica* y *Colobanthus quitensis*, las dos únicas especies herbáceas autóctonas que prosperan en la región de la Antártida marítima), estando dominado por vegetación criptogámica. Por otro lado, el gradiente latitudinal de temperatura (decreciente hacia el Polo Sur) es sin duda el factor abiótico más importante para la ecología de estas regiones. Al mismo tiempo, todas las zonas de estudio en esta tesis se caracterizan por presentar bajo contenido y disponibilidad de Nitrógeno (N) en el suelo, condiciones típicas de regiones de latitudes altas, lo cual limita la productividad primaria en estas zonas. La disponibilidad de N está condicionada por la entrada de este elemento en el ecosistema (fundamentalmente vía deposición atmosférica o fijación biológica) y por diferentes factores que determinan su acumulación o reciclado, entre los que se encuentra tanto la temperatura como los microorganismos del suelo. Del mismo modo, ambas regiones se encuentran amenazadas por los efectos del cambio climático, que se hace especialmente evidente en la región de la Antártida marítima y que ya está afectando a la composición y estructura de sus comunidades vegetales. Sin embargo, la escasez de estudios evaluando los principales mecanismos y factores ambientales que regulan la disponibilidad de N y el funcionamiento de los ecosistemas de la región de Tierra del Fuego o la Antártida impide anticipar las consecuencias que pueden desencadenar cambios en los patrones climáticos de ambas regiones. Por ello, urge desarrollar estudios que ayuden a comprender la respuesta de dichos ecosistemas frente tanto a los cambios ambientales que ya están afectando estas regiones como a los que aún están por venir.

Objetivos y resultados: La presente tesis doctoral pretende mejorar el conocimiento existente sobre el papel de las comunidades bióticas en el funcionamiento de los ecosistemas australes.

Más concretamente, se pretende evaluar las relaciones entre vegetación, comunidades microbianas y variables relacionadas con el ciclo del N en la región subantártica de Tierra del Fuego y la Antártida marítima. Los objetivos específicos son:

- Evaluar el papel de la simbiosis en el funcionamiento de los ecosistemas fueguinos, prestando especial atención a la especie herbácea *Gunnera magellanica*.
- Analizar la conexión entre la diversidad taxonómica de la vegetación, los principales factores ambientales y la estructura y composición de la comunidad microbiana del suelo en los ecosistemas australes.
- Identificar las relaciones entre la comunidad vegetal y los diferentes mecanismos implicados en el funcionamiento de los ecosistemas australes.

La presente tesis doctoral está organizada en cuatro capítulos, cuyos resultados más destacados son:

- La entrada de N vía fijación biológica es extraordinariamente alta en Tierra del Fuego, debido fundamentalmente a la abundancia de la especie *G. magellanica*. Además, los datos de $\delta^{15}N$ apuntan a que la vegetación no fijadora obtiene, de forma eficiente, grandes cantidades de N mediante el establecimiento de micorrizas (con una tasa estimada de transferencia del 80% del N capturado por el hongo hacia la planta hospedante), lo que permitiría a la vegetación desarrollarse en ausencia de condiciones limitantes de nutrientes.
- La diversidad y composición de la comunidad microbiana (múltiples taxones de arqueas, bacterias y hongos) en la región de Tierra del Fuego están asociadas a factores abióticos como la temperatura, el contenido de nutrientes o la humedad del suelo, pero también está estrechamente relacionada con atributos de la comunidad vegetal como son la riqueza de especies, su productividad primaria neta o la transición entre hábitats (de bosque de especies de *Nothofagus* a tundra alpina).
- Finalmente, la presencia de vegetación y comunidades de la costra biológica del suelo, en general, se asocia positivamente con la disponibilidad de nutrientes en suelo y la abundancia de microorganismos en la Antártida marítima, en comparación con áreas carentes de vegetación. Sin embargo, la identidad taxonómica de la vegetación condiciona el signo y magnitud de dicha asociación.

Conclusiones: Mediante los diferentes capítulos de esta tesis hemos podido obtener información relevante para comprender el funcionamiento de los ecosistemas australes. En

primer lugar, el establecimiento de relaciones simbióticas aparece como la causa potencial de la extraordinaria entrada de N en la región de Tierra del Fuego, así como de la eficiente captación y transferencia del N fijado en el ecosistema. La especie *G. magellanica*, ampliamente distribuida en la región, destaca como una especie crucial para el funcionamiento de los ecosistemas fueguinos, ya que su extraordinariamente alta actividad fijadora de N se mantiene a lo largo de su amplio rango ecológico. Por otro lado, la relación entre la vegetación y la comunidad microbiana en los ecosistemas australes de Tierra del Fuego y la Antártida marítima es muy estrecha, sugiriendo que cualquier alteración en la extensión y estructura de la vegetación como consecuencia del cambio climático podrían conllevar cambios drásticos en el funcionamiento de los ecosistemas terrestres en la región de Tierra del Fuego y la Antártida marítima.

GENERAL SUMMARY

Introduction: The present doctoral thesis, entitled "The nitrogen cycle in the Chilean sub-Antarctic region and the Maritime Antarctic. Major ecological drivers.", studies different aspects related to the functionality of the Antarctic and sub-Antarctic ecosystems. The study area includes the Tierra del Fuego region, located at the southernmost tip of the American continent, and the archipelago of the South Shetland Islands, included in the maritime Antarctic region and close to the Antarctic Peninsula. Both regions have similarities, but also hold important differences. For instance, both regions contrast in their different ecological characteristics even though they are located only 900 km away. The region of Tierra del Fuego hosts the southernmost forests on the planet, and therefore closer to the Antarctic continent which is characterized by the almost total absence of vascular plants (except for *Deschampsia antarctica* and *Colobanthus quitensis*, the only two native herbaceous species that thrive in the maritime Antarctic region), and the dominance of cryptogamic vegetation. Also, the latitudinal gradient of temperature (decreasing towards the South Pole) is undoubtedly the most important abiotic factor conditioning the ecology of these regions. At the same time, both regions are characterized by low contents and availability of nitrogen (N) in the soil, which often limits the primary productivity in high latitude regions. The availability of N is conditioned by the entry of this element into the ecosystem (mostly via atmospheric deposition or biological fixation) and by different factors (primarily temperature but also the microbial community of the soil) that determine its accumulation or cycling in the system. Moreover, both regions are threatened by the ongoing climate change, which is especially evident in the maritime Antarctic region (it has experience a large increase in temperature during the last decades which is already affecting the composition and distribution of plant communities). The scarcity of studies evaluating the main mechanisms and environmental factors that regulate the availability of N and the ecosystem functioning in the regions of Tierra del Fuego or Antarctica prevents anticipating the consequences of changes in the climatic patterns of both regions. Therefore, it is important to develop studies helping to understand the response of these ecosystems to ongoing climate change.

Objectives and main results: This doctoral thesis aims to improve our knowledge about the role of biotic communities in the functioning of the austral ecosystems. More specifically, it aims to evaluate the relationship between the vegetation and the microbial community and between them and different variables of the N cycle in the sub-Antarctic region of Tierra del Fuego and the maritime Antarctica. The specific objectives are:

- To evaluate the role of symbiosis in the functioning of Fuegian ecosystems, paying special attention to the herb *Gunnera magellanica*.
- To analyze the links between plant taxonomic diversity, major environmental factors and the diversity and composition of the soil microbial community in the Austral ecosystems.
- To identify the relationships between the plant community and the different mechanisms involved in the functioning of Austral ecosystems.

The present doctoral thesis includes four chapters, whose most important results are:

- The entry of N via biological fixation is extraordinarily high in Tierra del Fuego, mainly due to the abundance of the herb *G. magellanica*. In addition, the values of $\delta^{15}N$ shows that the non-fixing vegetation potentially obtains large amounts of N via the establishment of mycorrhizae (with an estimated transfer rate of about 80% of the N captured by the symbiotic fungus towards the host plant), allowing the vegetation to develop in the absence of nutrient limiting conditions.
- The diversity and composition of the microbial communities (multiple archaeal, bacterial and fungal taxa) in Tierra del Fuego are regulated by abiotic factors such as temperature, nutrient content or soil moisture, but it is also closely associated with attributes of the plant community such as species richness, net primary productivity or habitat change (from *Nothofagus* forest to alpine tundra). However, these relationships are different depending on the taxonomic group considered.
- Finally, the presence of vegetation (in general) is positively associated with soil nutrient availability and the abundance of microorganisms when compared to areas devoid of vegetation in maritime Antarctica. However, the taxonomic identity of the vegetation determines the sign and magnitude of this association.

Conclusions: Throughout the different chapters of this thesis I have been able to obtain relevant information to understand the functioning of austral ecosystems. Thus, the establishment of symbiotic relationships appears as the cause of the extraordinary entry of N into Fuegian ecosystems, as well as the efficient capture and transfer of the N fixed through the ecosystem. *Gunnera magellanica*, widely distributed in the region, seems a crucial species for the functioning of Tierra del Fuego ecosystems, since its extraordinarily high N-fixation activity is maintained throughout its wide ecological niche. The relationship between the vegetation and the microbial community in the ecosystems of Tierra del Fuego and Maritime Antarctica is very narrow, suggesting that any changes in the extension and structure of

vegetation communities as a consequence of climate change could lead to drastic changes in the functioning of these ecosystems.

INTRODUCCIÓN GENERAL

1 Ecosistemas antárticos y subantárticos: características e importancia

Los ecosistemas terrestres de latitudes altas en el hemisferio sur comprenden la Antártida continental y sus archipiélagos e islas adyacentes, las regiones subantárticas del extremo austral de Sudamérica (Tierra del Fuego) y múltiples islas y archipiélagos ubicados alrededor del Océano Antártico (Fig. 1). En el caso concreto de la zona de Tierra del Fuego, su inclusión dentro de la región subantártica[1] se debe fundamentalmente a criterios biogeográficos y ecológicos (Tuhkanen, 1992; Morrone, 2000; Moon *et al.*, 2017). Esta zona es la región continental más meridional y por lo tanto más próxima a la Antártida, de la que se encuentra separada por una franja de agua de apenas 900 km de ancho (Mar de Hoces o Paso de Drake). Estas dos regiones comparten algunas similitudes, pero también importantes diferencias. A nivel macroclimático, el gradiente latitudinal de temperatura (decreciente

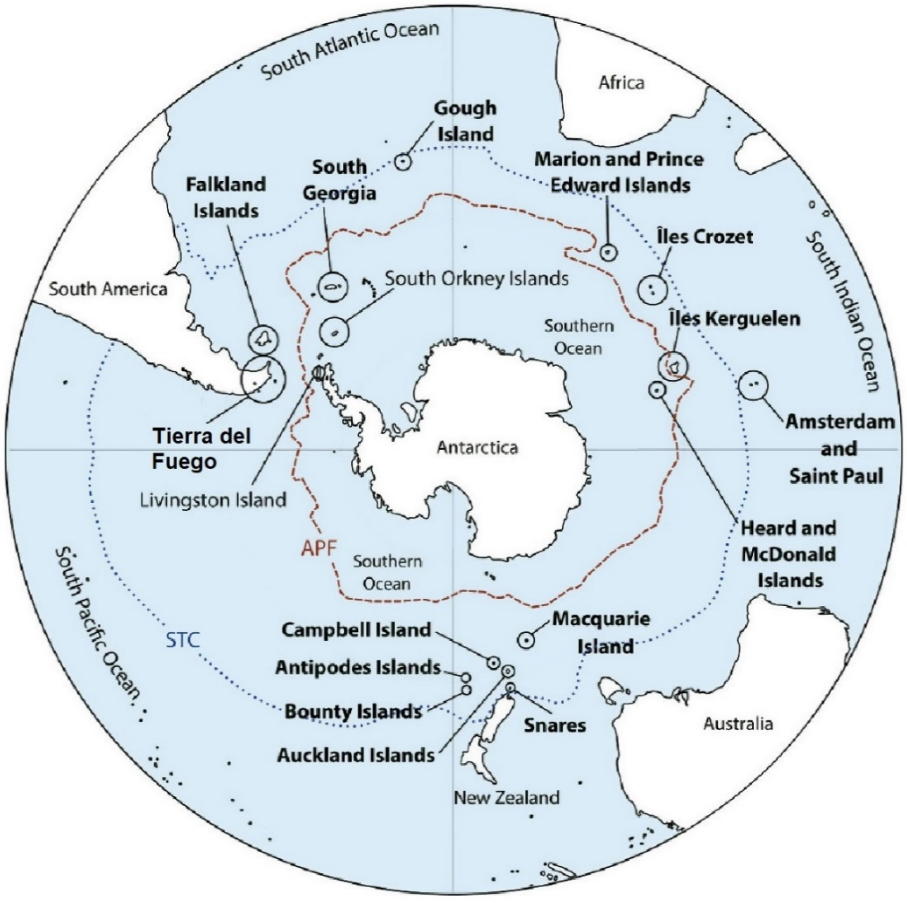


Figura 1: Mapa de la región austral indicando la extensión de la región subantártica, ubicada entre STC (Sub-Tropical Convergence) y APF (Antarctic Polar Front; modificado de Moon et al. 2017).

[1] Por región subantártica se entiende la región aislada entorno al Océano Antártico que se localiza entre la Convergencia Subtropical (STC) y el Frente Polar Antártico (APF; Moon et al. 2017).

hacia el polo) es sin duda el factor abiótico más importante para la ecología de estas regiones que, junto con los patrones regionales de precipitación y los fuertes vientos que soportan, determinan su variabilidad climática y biótica. Así, las regiones de Tierra del Fuego y la Antártida marítima contrastan enormemente en sus características ecológicas y biológicas, lo que permite utilizar dicho gradiente para estudiar estos ecosistemas y ayudar a discernir el papel de los factores ambientales en su funcionamiento.

La región noroccidental de la península antártica es la región más septentrional de este continente (Fig. 1, 2a). Las condiciones climáticas extremas de la Antártida se ven atenuadas en esta zona debido a una gran influencia oceánica, dando lugar a la región biogeográfica conocida como Antártida marítima (Benninghoff, 1987; Peat *et al.*, 2007). La Antártida marítima se caracteriza fundamentalmente por unas temperaturas más suaves que las presentes en el interior del continente. La tundra, bioma propio de las regiones polares, está aquí dominada por una vegetación eminentemente criptogámica, fundamentalmente compuesta por especies de líquenes y musgos (Fig. 3; Longton 1967; Green et al. 2007). Sin embargo, el clima algo más benigno en esta zona permite el crecimiento y desarrollo de las

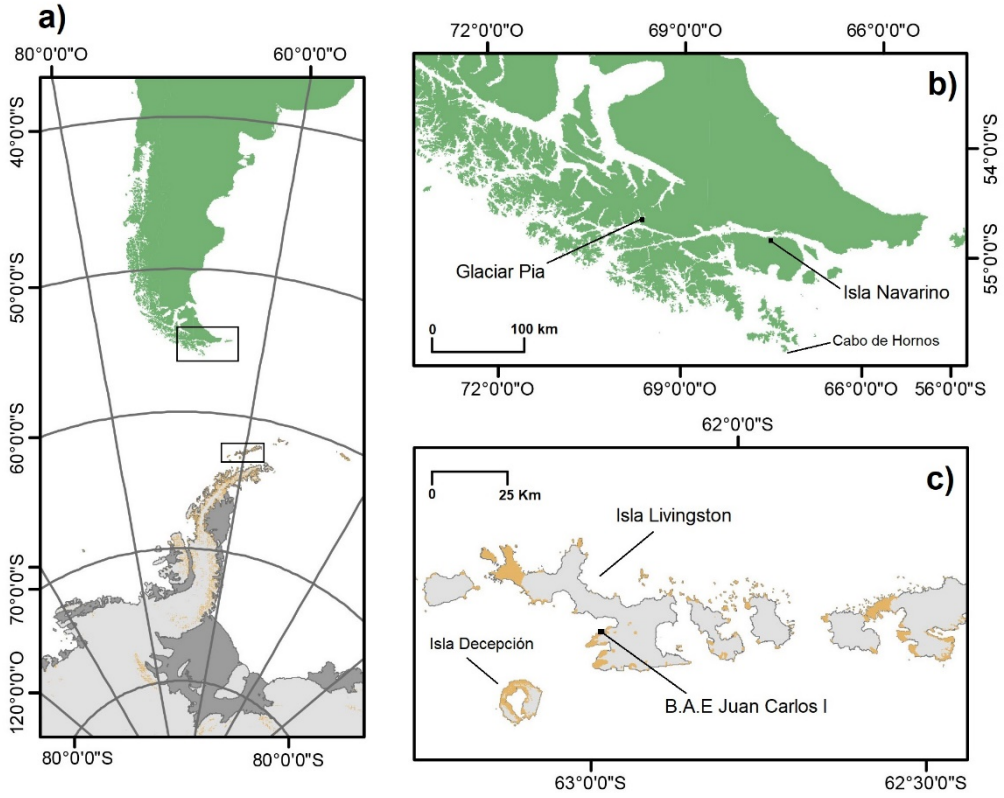


Figura 2: a) Detalle del Mar de Hoces con la ubicación de las dos zonas de estudio; b) detalle de la ubicación de Isla Navarino Tierra del Fuego (Chile); c) detalle de la ubicación de la Base Antártica Española Juan Carlos I (Isla Livingston, Archipiélago de las Shetland del Sur).

dos únicas especies de plantas vasculares presentes en la Antártida: *Deschampsia antarctica* É.Desv. y *Colobanthus quitensis* (Kunth) Bartl. (Fig. 3 c,f). La vegetación, relegada a aquellas zonas que quedan expuestas por el deshielo durante al menos una parte del año (Vieira *et al.*, 2014), se desarrolla sobre substratos rocosos en crestas, colinas, morrenas glaciares o en substratos detríticos o volcánicos en terrazas costeras. La vegetación criptogámica puede llegar a formar densos tapetes y costra biológica (Fig. 3a), de gran importancia para el funcionamiento de estos ecosistemas. Durante el verano austral, parte del hielo y la nieve acumulados durante el invierno se funde, permitiendo la colonización del substrato expuesto por múltiples organismos (Fig. 3e; Kennedy 1993; Bergstrom et al. 2006).

Los ecosistemas terrestres de la Antártida marítima se encuentran entre las regiones del planeta que han experimentado un mayor aumento de temperatura desde mediados del s. XX (Turner *et al.*, 2005). Aunque el incremento parece haberse invertido en algunas regiones durante los últimos años (Turner *et al.*, 2016), las consecuencias son ya evidentes en forma de retroceso glaciar o cambios en los patrones de distribución de la vegetación en los ecosistemas antárticos. Por ejemplo, la planta vascular *Deschampsia antarctica* ha experimentado un importante aumento en el número y extensión de sus poblaciones como consecuencia del calentamiento en algunas regiones de la Península Antártica (Torres-Mellado *et al.*, 2011; Cannone *et al.*, 2016). Asimismo, las comunidades de musgos muestran una mayor actividad biológica también en esta región (Amesbury *et al.*, 2017) y algunas especies de líquenes están comenzando a colapsar debido a la mayor innivación y acortamiento de la fase efectiva de crecimiento (Sancho *et al.*, 2017). De igual manera, el aumento de la temperatura, sumada a la intensa actividad humana en algunas zonas (Tin *et al.*, 2009; Hughes *et al.*, 2011), puede favorecer el establecimiento y reproducción con éxito de especies alóctonas en ecosistemas antárticos. Recientemente, varias especies habituales en la flora orófila de la región de Tierra del Fuego tales como *Nassauvia magellanica* o *Gamochaeta nivalis* fueron localizadas creciendo en Isla Decepción, en la Antártida marítima (Smith & Richardson, 2010). Ello enfatiza el riesgo de invasión de especies biogeográficamente próximas debido a que existen múltiples vectores de transporte de propágulos entre ambas regiones (Muñoz *et al.*, 2004; Chown *et al.*, 2012). Así, el funcionamiento de los ecosistemas antárticos está potencialmente amenazado por los cambios climáticos que están ocurriendo en la región y las alteraciones en las comunidades bióticas asociadas a los mismos, por lo que es crucial llevar a cabo estudios que permitan contextualizar estos cambios para poder predecir sus consecuencias ecológicas.

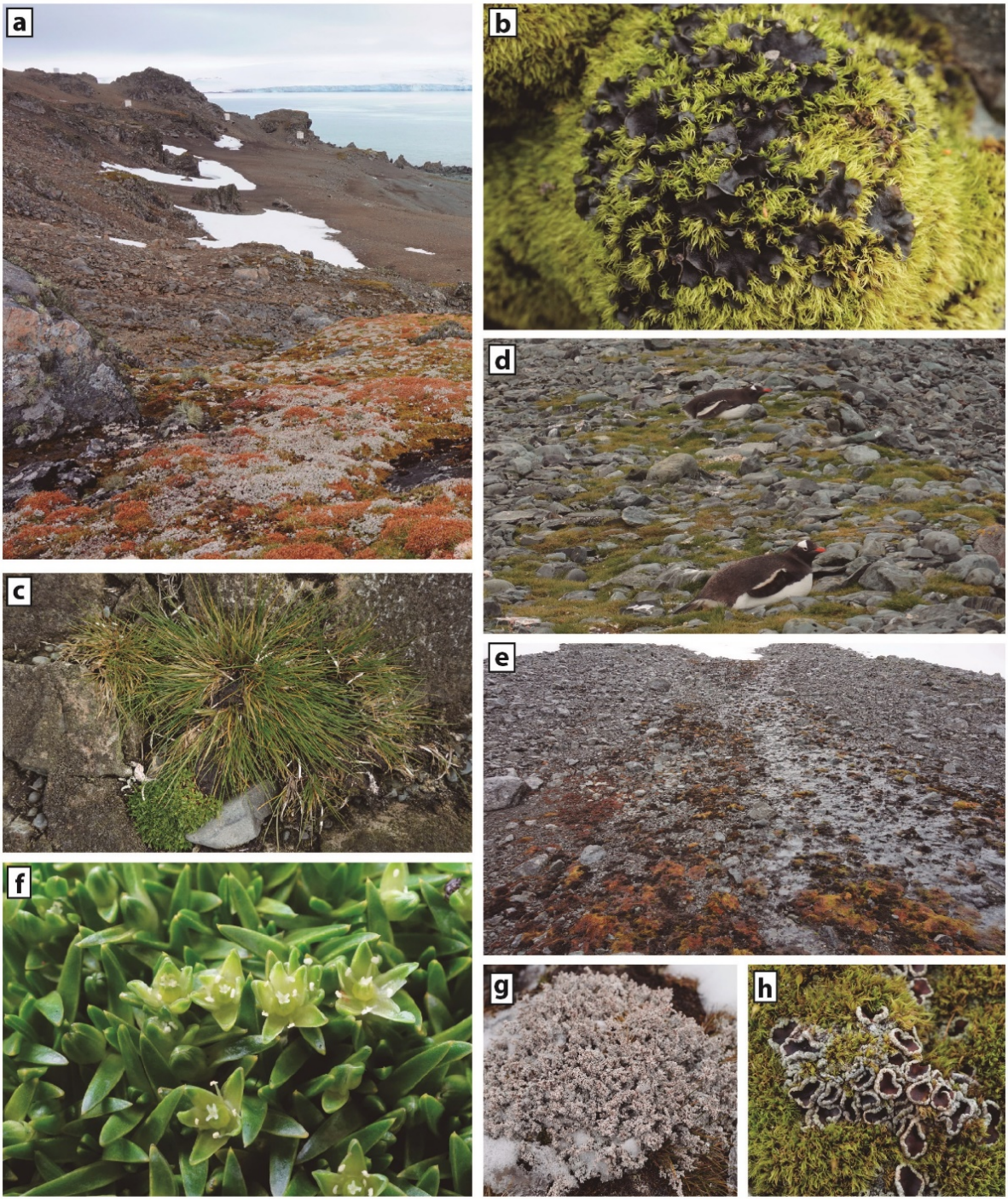


Figura 3: (a) Vista de una comunidad formada por criptógamas y plantas vasculares en las inmediaciones de la B.A.E. Juan Carlos I. (b) *Leptogium puberulum* sobre musgos; (c) *Deschampsia antarctica*; (d) Pingüinos Papúa (*Pygoscelis papua*) sobre comunidad de *Sanionia uncinata*; (e) Comunidad de *L. puberulum* en zona de escorrentía; (f) Detalle de *Colobanthus quitensis*; (g) *Stereocaulon alpinum*; (h) *Psoroma* sp.

A diferencia de lo que sucede en la región boreal, la transición entre los biomas de tundra y bosque en el hemisferio sur no sucede de forma progresiva ya que el Océano Antártico ocupa la extensión austral dónde debería producirse el reemplazo entre biomas. Sin embargo, una situación equiparable a la transición de la tundra hacia zonas boscosas sucede en la región subantártica de Tierra de Fuego, en el extremo sur del continente americano. La región subantártica de Tierra del Fuego (Fig. 1,2b) alberga las masas forestales más australes del mundo y, por lo tanto, más próximas a la Antártida (Molina *et al.*, 2016). Estos bosques (bosque subantártico magallánico; Fig. 4a) cubren una de las zonas más prístinas y mejor conservas del planeta debido a la baja intensidad de la actividad humana (Mittermeier *et al.*, 2003). Están formados fundamentalmente por especies del género *Nothofagus*, que incluyen tanto especies perennifolias (*Nothofagus betuloides* (Mirb.) Oerst.; Fig 4b) como caducifolias (*N. pumilio* (Poepp. & Endl.; Fig X) Krasser y *N. antarctica* (G.Forst.) Oerst.; Fig. 4c), que aparecen junto a otras especies arbóreas características como *Drimys winteri* J.R.Forst. & G.Forst. Además, esta región alberga una intensa actividad glaciar, pudiéndose encontrar aquí algunos de los campos de hielo y glaciares más extensos del planeta. Uno de los aspectos climáticos más característicos de la región de Tierra del Fuego es el abrupto gradiente de precipitación existente entre la costa oeste, que recoge los vientos húmedos del Pacífico (~2000 mm anuales de precipitación) y la costa este o atlántica con escasa precipitación por efecto de sombra de lluvia (~400 mm) donde encontramos ecosistemas áridos como la estepa, también denominada pampa (Fig. 4g; Godley 1960; Tuhkanen 1992; Santana et al. 2006). En las estribaciones más meridionales de esta región (archipiélago de Cabo de Hornos; Fig. 4f), así como en las zonas de mayor precipitación (fachada pacífica), la vegetación arbórea se encuentra excluida o reducida dando lugar a la tundra subantártica (Fig. 4f). En estas zonas aparecen especies arbustivas (*Gaultheria mucronata* (L.f.) Hook. & Arn. o *Empetrum rubrum* Vahl ex Willd.) y formas enanas de especies del género *Nothofagus*, junto con multitud de briófitos y líquenes (Fig. 4e). Las condiciones climáticas que limitan el crecimiento de especies arbóreas en los polos ocurren de manera análoga en las cumbres montañosas de Tierra del Fuego (Körner, 2003; Molina *et al.*, 2016). Debido a la disminución progresiva de la temperatura con respecto a la altitud, junto con los fuertes vientos que soportan estas regiones, en un determinado punto las especies arbóreas adoptan un porte arbustivo (*krummholz*) y luego van dando paso a especies de menor porte junto con especies criptógamas (Fig. 4g). Así, la baja temperatura es el factor que define estos ecosistemas alpinos en general, limitando no sólo la presencia de determinados biotipos, sino también su productividad primaria y los procesos biogeoquímicos que sustentan el ecosistema. Es

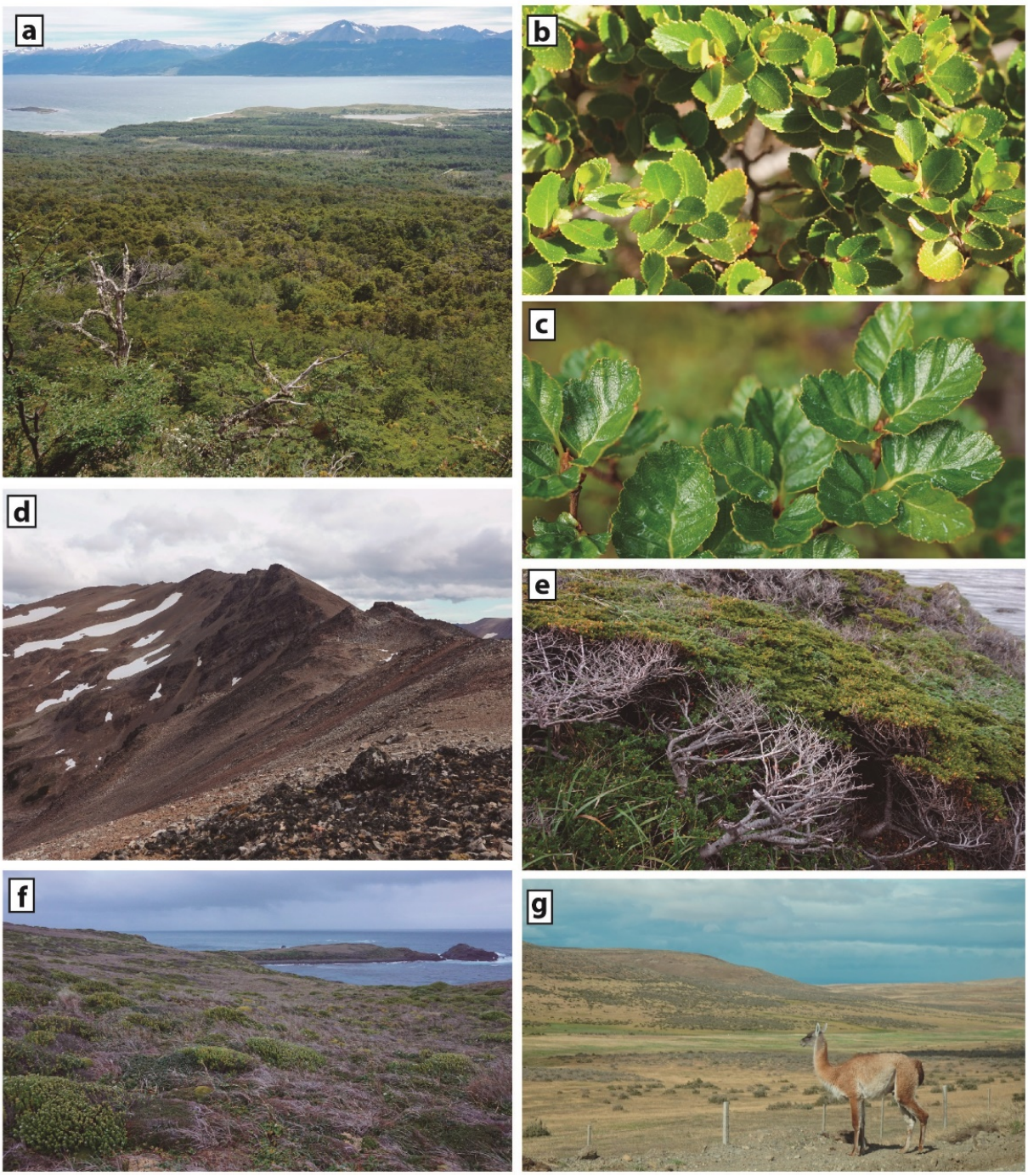


Figura 4: (a) Vista del bosque subantártico mixto en Isla Navarino. (b) *Nothofagus betuloides,* detalle; (c) *N. antractica,* detalle; (d) Tundra altoandina dominada por especies criptógamas en Isla Navarino; (e) Formación en cojín de *N. betuloides* típica de la tundra subantártica en el Archipiélago del Cabo de Hornos; (f) Vista general de la tundra subantártica en Cabo de Hornos (g) Vista de la zona árida de Tierra del Fuego (pampa), en Isla Grande de Tierra del Fuego ubicada al este del Canal Beagle.

remarcable la conexión biogeográfica entre el continente antártico y la alta montaña de Tierra del Fuego, pues comparten un gran número de especies de criptógamas presentes a ambos lados del Mar de Hoces (Øvstedal & Smith, 2001; Park *et al.*, 2018).

La región subantártica se compone de ecosistemas frágiles y singulares que también están experimentando los efectos del cambio climático (Whinam *et al.*, 2006; Bergstrom & Selkirk, 2007; Selkirk, 2007; Iriarte *et al.*, 2010). Los glaciares fueguinos en su mayoría han retrocedido drásticamente en las últimas décadas y continúan haciéndolo en la actualidad (Melkonian *et al.*, 2013). El aumento de la temperatura en la región puede favorecer la expansión altitudinal del bosque subantártico en detrimento de la tundra alto-andina (Harsch *et al.*, 2009), alterar la distribución y composición de la vegetación (Cornelissen *et al.*, 2001) o modificar los ciclos biogeoquímicos y la disponibilidad de nutrientes (Aerts, 2006). Sin embargo, muy poco se conoce sobre los factores bióticos y abióticos que potencialmente controlan el funcionamiento de los ecosistemas fueguinos, por lo que enriquecer este conocimiento es crucial para poder predecir con más exactitud el futuro en esta región bajo los distintos escenarios de cambio global.

3.2 Importancia del nitrógeno en la región subantártica chilena y la Antártida marítima

El N es uno de los elementos más importantes para la vida, estando considerado como uno de los factores más limitantes para la productividad primaria de los ecosistemas terrestres a nivel global (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008). Además, se considera que las regiones de latitudes altas (y regiones alpinas) presentan un contenido y disponibilidad de N inferior al de zonas ecuatoriales o de latitudes medias (Hedin *et al.*, 2009). Esto se debe a que el contenido y la disponibilidad de nitrógeno en los ecosistemas terrestres están íntimamente relacionados con la actividad biológica (p. ej. fijación de N atmosférico), así como con la diversidad (funcional y taxonómica) y composición de las comunidades microbianas y de plantas (Hooper & Vitousek, 1997; Tatarko & Knops, 2018), y dependen en última instancia de factores abióticos como la temperatura (Myers, 1975; Brookshire *et al.*, 2011). Así, la disponibilidad de N tiende a disminuir a medida que aumenta la latitud, y por ello es esperable que las regiones de Tierra del Fuego y la Antártida marítima presenten *a priori* escasa disponibilidad de N, estando su productividad limitada por este nutriente. Sin embargo, la mayoría de estudios en latitudes altas se han desarrollado en el hemisferio norte y son escasos los estudios sobre los principales mecanismos y factores ambientales que

regulan la disponibilidad de N y el funcionamiento de los ecosistemas de la región de Tierra del Fuego o la Antártida (Mazzarino *et al.*, 1998). Dichos estudios se han centrado en la descripción y cuantificación de procesos concretos como la descomposición y la mineralización (Caldentey et al. 2001; Frangi et al. 2005) y, pese al creciente interés a nivel global, el papel de las comunidades microbianas y de las interacciones planta-microorganismo en el funcionamiento de los ecosistemas australes permanece prácticamente inexplorado (Fernández-Martínez *et al.*, 2016, 2017).

La entrada de nitrógeno en un ecosistema puede deberse a procesos de origen abiótico (p. ej. deposición atmosférica) o biótico (fijación biológica de nitrógeno, FBN). La deposición atmosférica de N es un fenómeno que, pese a que se produce de forma natural asociado a la actividad eléctrica en la atmósfera (Drapcho *et al.*, 1983), hoy en día está mayoritariamente relacionado con la actividad humana (Dentener *et al.*, 2006). En este sentido, la región subantártica chilena y, en mayor medida, la Antártida marítima se encuentran entre las zonas con menor tasa de deposición de N del mundo, < 1 kg N · ha^{-1} · $año^{-1}$, muy por debajo de los > 20 kg N · ha^{-1} · $año^{-1}$ en algunas regiones altamente industrializadas del hemisferio norte (Dentener *et al.*, 2006). Así, la FBN se aparece como la fuente fundamental de N en estas regiones. La FBN es un proceso exclusivamente desarrollado por microorganismos, tanto de vida libre como en simbiosis, y consiste en la reducción del N_2 atmosférico a NH_4^+, proceso mediado por una enzima (nitrogenasa), por lo que es muy dependiente de la temperatura (Gibson, 1962; Gibson & Jordan, 1983; Gundale *et al.*, 2012). La capacidad de fijar N directamente de la atmósfera confiere una ventaja adaptativa a las plantas que establecen simbiosis con estos microorganismos con respecto a las plantas que se ven obligadas a captar el N del suelo (Houlton *et al.*, 2018). Así, la FBN debería en teoría verse potenciada por una baja disponibilidad de N. Sin embargo, las estimaciones globales de FBN establecen mayores tasas en los trópicos y menor fijación en ecosistemas de latitudes altas como consecuencia fundamentalmente de las bajas temperaturas y menor productividad primaria (Vitousek *et al.*, 2002; Houlton *et al.*, 2008; Menge *et al.*, 2014), pero también por la menor diversidad y abundancia de grupos como las leguminosas (Menge *et al.*, 2014; Menge & Crews, 2016). La FBN ha sido intensamente estudiada y cuantificada en ecosistemas boreales, mientras que existe un gran desconocimiento sobre el papel de la fijación en el funcionamiento de los ecosistemas más meridionales del planeta. Esta situación sorprende ya que pese a que el hemisferio sur es el territorio natural del único género de angiospermas que establece simbiosis con

cianobacterias (*Gunnera*, ver apartado 3.3) se ha excluido de todas las estimaciones globales de FBN realizadas hasta la fecha (Cleveland *et al.*, 1999; Galloway *et al.*, 2004). Mientras la baja productividad de la Antártida marítima y sus características ecológicas concuerdan con las teorías generalmente aceptadas para estos ecosistemas, recientes estudios parecen contravenir la aplicación de las mismas en la región de Tierra del Fuego, pues presentan algunas de las tasas de crecimiento más altas del planeta en ciertos líquenes, así como un gran éxito de la vegetación en la colonización de morrenas glaciares (Sancho *et al.*, 2011; Arróniz-Crespo *et al.*, 2014).

Además de la entrada de N al medio, la disponibilidad de este nutriente depende también de otros factores que condicionan su acumulación en el suelo. Paradójicamente, la acumulación de N sigue un patrón latitudinal opuesto a su disponibilidad, siendo mayor en ecosistemas fríos debido a la acumulación de materia orgánica es estos ecosistemas (Post *et al.*, 1985). Los procesos biológicos que intervienen en la degradación de la materia orgánica (estos son la mineralización y descomposición) están mediados fundamentalmente por la comunidad microbiana y dependen de factores como la temperatura y la humedad del medio, así como de la composición química de la materia orgánica (Leiros *et al.*, 1999; Hobbie *et al.*, 2000b; Berg & McClaugherty, 2014). La temperatura es quizá el factor más influyente para estos procesos ya que condiciona directamente la termodinámica de las reacciones químicas implicadas y la actividad enzimática (energía de activación, cinética de reacción, etc.; Hobbie et al. 2000; Davidson and Janssens 2006). En ecosistemas de latitudes altas, las bajas temperaturas ralentizan las tasas de descomposición y mineralización, y como consecuencia fomentan la acumulación de la materia orgánica que incluye la mayor parte del N y C (orgánico) en el suelo (Robinson, 2002). La humedad del suelo también condiciona la actividad microbiana y la degradación de la materia orgánica en regiones de latitudes altas (Hobbie *et al.*, 2000b; Robinson, 2002). En estas regiones, la humedad del suelo no se considera un factor limitante para la actividad biológica, dada la escasa evapotranspiración (Bliss *et al.*, 1981). Además, pese a que las bajas temperaturas hacen que el agua del suelo se congele temporal o permanentemente en algunas zonas, una fracción del agua se mantiene en estado líquido dentro de la microestructura del suelo, permaneciendo disponible para los microorganismos (Öquist *et al.*, 2009). Por último, la naturaleza química de la materia orgánica, esto es su composición química y su contenido en nutrientes, también afecta a la acumulación y disponibilidad de N en el medio. Por ejemplo, una baja concentración de carbohidratos solubles o un alto contenido en lignina ralentizan la descomposición (Austin &

Ballaré, 2010; Rahman *et al.*, 2013), siendo más fácil la descomposición de detritus de especies caducifolias que de especies perennifolias, y la de ambas más fácil que la de líquenes o briófitos (Hobbie *et al.*, 2000b; Lang *et al.*, 2009).

Estudiar las conexiones entre la vegetación y la comunidad microbiana, y de ambas con los principales mecanismos implicados en el funcionamiento de estos ecosistemas ayudará a paliar el gran vacío en nuestro conocimiento sobre estas cuestiones en la región de Tierra del Fuego y la Antártida marítima. Asimismo, los cambios que pueden experimentar estas regiones (aumento de temperatura, cambios en los patrones de precipitación, cambios en la composición y actividad biológica; (Bergstrom & Selkirk, 2007; Pendlebury & Barnes-Keoghan, 2007; Cabré *et al.*, 2016; Lee *et al.*, 2017) urgen a estudiar estos ecosistemas para poder evaluar su respuesta a nivel regional y global.

3.3 Papel de la flora y los microorganismos en el funcionamiento de los ecosistemas antárticos y subantárticos, con especial énfasis en la simbiosis

El funcionamiento de un ecosistema (su capacidad de realizar múltiples procesos biológicos, químicos y físicos de manera simultánea) depende directamente de la comunidad biótica que lo integra. Numerosos estudios realizados en las últimas décadas han puesto de manifiesto como la diversidad taxonómica y funcional de la vegetación impacta positivamente en procesos como la productividad, el reciclado de nutrientes y la fijación de carbono (Maestre *et al.*, 2012b; Tilman *et al.*, 2014; Gross *et al.*, 2017). Múltiples especies pueden compartir un determinado rasgo funcional, por lo que en ocasiones se les agrupa en gremios o grupos funcionales (Pla *et al.*, 2012). En el caso de la vegetación, determinados rasgos morfológicos o fisiológicos como, por ejemplo, el contenido en nitrógeno en los tejidos fotosintéticos, sus tasas de respiración o el establecimiento de relaciones simbióticas con otros organismos, tienen implicaciones directas sobre los procesos biogeoquímicos (Funk *et al.*, 2017). Lo mismo sucede con las comunidades microbianas del suelo, fundamentalmente a través de su capacidad para sintetizar determinadas enzimas o mediar en etapas clave de los ciclos biogeoquímicos. El establecimiento de relaciones simbióticas es un rasgo funcional especialmente importante, que confiere a la planta hospedante, entre otras ventajas, un aumento de su capacidad de obtención de nutrientes (Arora, 2013; Johnson *et al.*, 2016). Ello permite a estas especies poder competir de forma eficiente con otros organismos por dichos recursos. Por todo ello, el estudio de la diversidad funcional de la vegetación puede aportar información de especial relevancia para nuestra compresión sobre el funcionamiento de los

ecosistemas de las regiones de Tierra del Fuego y la Antártida marítima, lugares donde esta aproximación ha sido escasamente aplicada hasta la fecha (Barrera *et al.*, 2000; Fernández-Martínez *et al.*, 2016).

3.3.1 Simbiosis y fijación de nitrógeno

La fijación de N es llevada a cabo en exclusividad por procariotas (denominados microorganismos diazótrofos) con la capacidad de sintetizar la enzima nitrogenasa (Kim & Rees, 1994; Zhang *et al.*, 2016), principal vía natural de entrada de N en los ecosistemas (Gruber & Galloway, 2008). Los organismos diazótrofos en ecosistemas terrestres pueden ser de vida libre o simbiontes con plantas vasculares, hongos o briófitos (Stewart, 1969; Rai *et al.*, 2002; Santi *et al.*, 2013). En cuanto a la simbiosis con plantas vasculares, existen varios tipos de asociación (ver revisión Santi et al. 2013). Uno de los grupos más relevantes y más intensamente estudiados es el género *Rhizobium* (α-Proteobacteria) que se asocia fundamentalmente con miembros de la familia Fabaceae. La fijación de N por leguminosas es muy importante en las zonas tropicales y templadas pero disminuye considerablemente en las regiones de latitudes altas (Houlton *et al.*, 2008; Menge *et al.*, 2014). Otro grupo fundamental es el género *Frankia* (Actinobacteria) que se asocia con múltiples géneros de plantas a las que se denomina plantas actinorrícicas. La fijación de N por estas plantas es muy relevante en la región boreal, donde miembros de los géneros *Alnus* y *Dryas* son clave en la entrada de N al medio durante la sucesión primaria tras el retroceso glaciar (Kohls *et al.*, 1994, 2003). Por último, las cianobacterias son un grupo de microorganismos clave para el funcionamiento de ecosistemas extremos (Zakhia *et al.*, 2008; Whitton, 2012), en gran medida debido a su papel como fijadoras de N (Vitousek *et al.*, 2002). Las cianobacterias fijadoras de N pueden ser de vida libre o vivir en simbiosis con hongos (formando líquenes), musgos, antoceros, helechos (*Azolla*) y plantas vasculares (Cycadaceae y Gunneraceae; Rai et al. 2002).

El género *Gunnera* es el único miembro de la familia Gunneraceae y el único grupo conocido de plantas angiospermas que establece una relación endosimbionte con cianobacterias filamentosas del genero *Nostoc* (Osborne & Bergman, 2009). Esta asociación confiere a los miembros del género *Gunnera* la capacidad de obtener N directamente de la atmósfera mediante la actividad de la enzima nitrogenasa. El proceso por el cual se produce la infección de la planta, así como las adaptaciones fisiológicas que experimentan ambos miembros, han sido ampliamente tratados en la literatura (Silvester & McNamara, 1976; Johansson & Bergman, 1992; Rasmussen *et al.*, 1994; Bergman *et al.*, 1996; Uheda, 2001; Bergman, 2002). Con esta relación simbiótica la cianobacteria amplía su nicho ecológico al

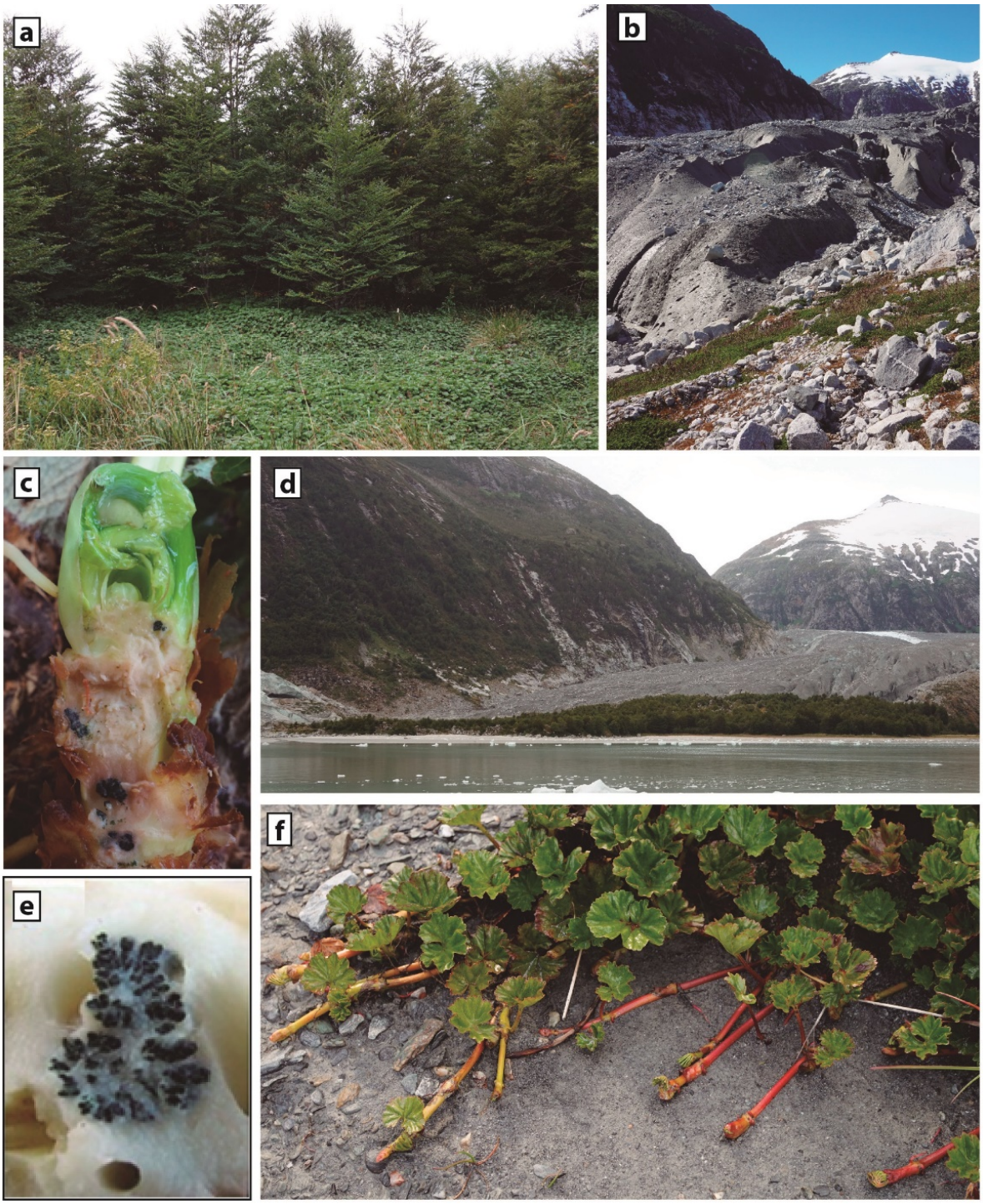


Figura 5: (a) Bosque de *Nothofagus antarctica* y *N. betuloides* en la morrena del Glaciar Pía con abundante cobertura de *Gunnera magellanica* en el estrato herbáceo; (b) *G. magellanica* colonizando las zonas recientemente expuestas frente al glaciar; (c) Sección transversal del rizoma de *G. magellanica* mostrando las colonias de *Nostoc* sp. (d) Vista de la sucesión primaria frente al Glaciar Pía; (e) Detalle de una colonia de *Nostoc* sp. en el rizoma de *G. tinctoria*; (f) Hábito de *G. magellanica* mostrando la producción de estolones.

mismo tiempo que la planta obtiene una ventaja competitiva con respecto a otras especies que no pueden fijar N atmosférico. Los miembros de la familia Gunneraceae se encuentran actualmente (sin considerar la distribución alóctona de varios taxones) distribuidos principalmente por el hemisferio sur, desde el paralelo 20 ºN pasando por la zona ecuatorial hasta las regiones más australes de Sudamérica y Nueva Zelanda (Wanntorp & Wanntorp, 2003; Wanntorp *et al.*, 2004). Está integrado por algo menos de 70 especies, de las cuales sólo dos se encuentran en la región austral de Sudamérica (Patagonia y Tierra del Fuego): *Gunnera magellanica* Lam. y *Gunnera lobata* Hook.f. (Wanntorp & Wanntorp, 2003). Ambas especies se encuentran filogenéticamente separadas del resto de especies del género, formando el subgénero *Misandra*, restringido al sur de la Patagonia. *Gunnera magellanica* es una hierba rizomatosa (Fig 5), con un amplio nicho ecológico y muy abundante en Tierra del Fuego, donde forma densas poblaciones sobre substratos alterados (morrenas) y en el sotobosque (Fig. 5 a,b). Tanto su abundancia como su capacidad para fijar N permiten suponer que *G. magellanica* sea una especie clave para el funcionamiento de los ecosistemas fueguinos. Sin embargo, muy pocos estudios han abordado sus características funcionales (Söderbäck *et al.*, 1990; Rousseaux *et al.*, 2001; Giordano *et al.*, 2003), y apenas existen trabajos evaluando su papel en el funcionamiento de los ecosistemas de Tierra del Fuego (Rousseaux *et al.*, 2001; Giordano *et al.*, 2003; Troncoso *et al.*, 2013; Pérez *et al.*, 2014).

Otro rasgo funcional muy importante relativo a la simbiosis es el establecimiento de micorrizas. Este fenómeno (asociación entre una planta y un hongo) es prácticamente universal en las plantas terrestres y tiene importantes implicaciones ecológicas (Malloch *et al.*, 1980; Van Der Heijden *et al.*, 2008; Faucon *et al.*, 2017). Mediante el establecimiento de micorrizas las plantas mejoran su estatus nutricional, pues les permite aumentar su capacidad de obtener agua y nutrientes (Arora, 2013). Una ventaja directa de la asociación consiste en el aumento de la superficie de captación de nutrientes clave como son el fósforo y formas orgánicas de N (la superficie del sistema radicular más la superficie propia del hongo micorrizado). Estos hongos cuentan con una serie de rasgos funcionales que les confieren la capacidad de movilizar nutrientes de substratos orgánicos e inorgánicos (Bolan, 1991; Johnson *et al.*, 2016). Así, el establecimiento de micorrizas aparece como una cuestión fundamental en ecosistemas donde la disponibilidad de nutrientes es baja, como ocurre en ecosistemas de latitudes altas. De hecho, se ha demostrado que las micorrizas juegan un papel fundamental en la captación de formas orgánicas de N por parte de especies vegetales en bosques boreales (Näsholm *et al.*, 1998, 2008), por lo que la planta puede así, en cierta

medida, cortocircuitar la descomposición de la materia orgánica y captar el N que se encuentra en el suelo en formas *a priori* no disponibles. Si bien el establecimiento de micorrizas no es necesario para captar eficientemente formas orgánicas de N (Hill *et al.*, 2011), esta simbiosis lo aumenta exponencialmente (Näsholm *et al.*, 2008).

3.4 Conectividad ecológica

Los escasos 900 km del Mar de Hoces (o Paso de Drake) separan estas dos zonas ecológicamente muy diferentes pero con importantes nexos de unión. Pese al abrupto contraste en las condicionas bióticas de ambas regiones, existe una intensa conectividad funcional y ecológica entre ellas. Desde un punto de vista geológico y geográfico, las estribaciones más australes de la Cordillera de los Andes se sumergen progresivamente dando origen al archipiélago de Tierra del Fuego, para emerger de nuevo al otro lado del Mar de Hoces y dar lugar a la Cordillera de los Antartandes, que vertebra la Península Antártica y continúa hacia el interior del continente. Desde un punto de vista biótico, comparten especies vegetales (principalmente las zonas alpina o costera de Tierra del Fuego y la Antártida marítima) como por ejemplo las dos únicas especies de plantas vasculares nativas de la Antártida (*D. antarctica* and *C. quitensis*, Fig. 2c,h; Moore 1983) o múltiples especies de líquenes y briófitos (Bednarek-Ochyra 2000; Ochyra et al. 2008), forman parte de las rutas migratorias de aves (Brown *et al.*, 1975), y múltiples mecanismos de dispersión de especies (viento, corrientes marinas, actividad humana) aseguran la conectividad entre ambas regiones (Muñoz *et al.*, 2004; Barnes *et al.*, 2006; Moon *et al.*, 2017). Finalmente, desde un punto de vista climático (o biogeográfico) las condiciones climáticas que encontramos en las cumbres de Tierra del Fuego no distan de las que podemos encontrar en las zonas costeras de la Península Antártica. Por ejemplo, la temperatura media anual en la zona alpina de Isla Navarino (Chile, Fig. 2b) ronda los 0,2 ºC, mientras que en Isla Livingston oscila entre 0 y -2 ºC (Fig. 2c). Además, se prevé un considerable aumento de la temperatura media y la precipitación en la Península Antártica, lo que previsiblemente conducirá a una disminución de la superficie actualmente cubierta por el hielo (Anisimov *et al.*, 2007; Lee *et al.*, 2017). Así, cabe plantearse un posible desplazamiento meridional, hacia la Antártida, de los ecosistemas terrestres de Tierra del Fuego, de manera análoga al avance del matorral y el bosque boreal hacia el Ártico en detrimento de la tundra (Myers-Smith *et al.*, 2011; Zhang *et al.*, 2013b). En tal caso, conocer el funcionamiento de los ecosistemas australes, incluyendo las relaciones entre la vegetación y la comunidad microbiana y éstas a su vez con los principales factores

ambientales (temperatura, disponibilidad de nutrientes, etc.) permitirá predecir con mayor precisión los impactos potenciales del cambio en estos ecosistemas y sus servicios.

3.5 Estructura de la tesis y objetivos generales

El objetivo general de esta tesis es evaluar las relaciones entre vegetación, comunidades microbianas y variables del ciclo del N en la región subantártica de Tierra del Fuego y la Antártida marítima. La presente tesis consta de cuatro capítulos, dedicados cada uno de ellos a abordar aspectos clave en el funcionamiento de los ecosistemas fueguinos y antárticos que apenas han sido estudiados hasta la fecha.

En el Capítulo 1 se evalúan los mecanismos de entrada y transferencia de N en una sucesión primaria. Este capítulo se centra en el papel de la vegetación y en concreto de la simbiosis que establecen diferentes especies de plantas con microorganismos como moduladores de la disponibilidad de N y su papel sustentador la alta productividad primaria observada en la morrena del glaciar Pía (Tierra del Fuego). Con este capítulo pretendemos contextualizar la importancia de la simbiosis microbiana para estos ecosistemas y conocer la relevancia de *G. magellanica* como fijador de N.

En el Capítulo 2 se estudia la diversidad funcional de *G. magellanica* a lo largo de un gradiente altitudinal. Con este capítulo pretendemos conocer la variabilidad morfológica y fisiológica de esta especie a lo largo de un gradiente altitudinal como aproximación al estudio de la amplitud de su nicho ecológico. Dada la relevancia de *G. magellanica* para el funcionamiento del ecosistema durante la sucesión primaria, este estudio pretende conocer si su relevancia como fuente de N se presume constante en esta región a pesar de la variabilidad de ecosistemas en Tierra del Fuego.

En el Capítulo 3 se estudian los patrones altitudinales de las comunidades microbianas en los suelos de Tierra del Fuego y su conexión con las etapas clave del ciclo del N. En concreto, analizamos cómo varía la diversidad y abundancia de diferentes grupos taxonómicos y funcionales de microorganismos e intentamos conocer los principales factores bióticos y abióticos que controlan dichos patrones. Además, evaluamos cómo los cambios observados en la comunidad microbiana se relacionan con los valores de diferentes variables y funciones relacionadas con la disponibilidad y reciclado de nutrientes.

En el Capítulo 4 se analizan las conexiones existentes entre la diversidad taxonómica de la vegetación y múltiples funciones del suelo en la Antártida marítima. En concreto,

estudiamos cómo afecta la vegetación y la cobertura de costra biológica al contenido de nutrientes del suelo y cómo su composición taxonómica se asocia a unos niveles diferentes de las funciones analizadas. Con este estudio, se pretende conocer cómo distintas especies de plantas vasculares, musgos y líquenes afectan a la funcionalidad de los ecosistemas antárticos.

CAPÍTULO 1: High nitrogen contribution by *Gunnera magellanica* and nitrogen transfer by mycorrhizae drive an extraordinarily fast primary succession in Sub-Antarctic Chile

Abstract

Recently exposed soils from the retreating Pia Glacier (Tierra del Fuego, Chile) are initially nutrient-depleted, but quickly turn into a fertile habitat. After just 34 years of exposure, total soil nitrogen content has increased from near zero to 1.5 % (DW basis) and a *Nothofagus* dominated forest is in place with trees reaching 10 m in height. Despite the lack of abiotic nitrogen inputs to this pristine region, the trees and other plants show no signs of nitrogen limitation, suggesting another source such as biological nitrogen fixation. Diazotrophic organisms, highly abundant across the succession, include the deciduous perennial herb *Gunnera magellanica* thriving profusely at almost all stages of the succession. Here, we simultaneously evaluated the N-fixation and the photosynthetic activity of dominant species along the chronosequence in front of Pia Glacier. We also evaluated the nitrogen isotope discrimination ratio ($\delta^{15}N$) in foliar tissue to better understand the fate of fixed N throughout the succession and asses the role of mycorrhizae in such a successful colonization. We found that *G. magellanica* may be considered as a N pump to the succession, with some of the highest nitrogenase activities reported exceeding an estimated contribution of 300 kg $N \cdot ha^{-1} \cdot yr^{-1}$. Conversely, photosynthetic activity values were in accordance with generally reported rates in similar regions. $\delta^{15}N$ analysis showed a potentially efficient N transfer in the system, with about 80 % of N taken from the fungi transferred to the host plant. Altogether, our results suggest plant-microbial symbioses are a key processes in Tierra del Fuego ecosystems both by supplying N via the exceptional cyanobacterial symbiosis in *G. magellanica* and by allowing a highly efficient nutrient transfer system via mycorrhizae, both sustaining this rapid and successful primary colonization.

Keywords: Cyanobacteria, nitrogenase activity, nitrogen isotope discrimination, photosynthesis, symbiosis, Tierra del Fuego.

Introduction

Recently exposed soils after glacier retreat are often considered nitrogen (N) limited habitats (Yoshitake *et al.*, 2007; Göransson *et al.*, 2011; Schulz *et al.*, 2013). Although ancient and allochthonous nutrient stocks may support heterotrophic communities in immediately exposed barren substrates (Bardgett *et al.*, 2007; Sattin *et al.*, 2010; Guelland *et al.*, 2013b), autotrophic and diazotrophic pioneer organisms trigger ecological succession by progressively increasing soil organic matter (Matthews 1992; Nakatsubo et al. 2005; Schmidt et al. 2008). The colonization dynamics of microbial (Knelman *et al.*, 2012; Fernández-Martínez *et al.*, 2017) and plant (Whittaker, 1993; Raffl *et al.*, 2006) communities as well as some parallel shifts in soil nutrient cycling (e.g. soil respiration and mineralization; Strauss et al. 2012; Guelland et al. 2013a, b) have been extensively studied. However, the quantification of the photosynthetic and diazotrophic performance of pioneer vegetation and microorganisms associated across succession has been barely studied, despite of its paramount importance to accurately characterize the impact of vegetation colonization on soil nutrient availability and cycling.

Ecological succession is generally constrained (Tilman, 1990), with nutrient availability (especially N in young soils and phosphorus (P) in old soils) as the most common limiting factors for ecological succession (Vitousek *et al.*, 1989, 2010; Vitousek & Howarth, 1991; Yoshitake *et al.*, 2007; Batterman *et al.*, 2013; Schmidt *et al.*, 2016; Castle *et al.*, 2017). On surfaces exposed by retreating glaciers, N limitation often occurs in early stages of colonization and is progressively mitigated by the increasing quantity and availability of N in the system coming from deposition and biological processes (Vitousek & Farrington, 1997). As atmospheric deposition is small for most extensive glacier forelands excepting Himalayas (i.e. < 1 kg N $\cdot$ ha^{-1} $\cdot$ yr.$^{-1}$; Dentener et al. 2006), the primary source of N at early stages of glacier succession is potentially biological N fixation (LeBauer & Treseder, 2008), and bedrock erosion to a lesser extent (Houlton *et al.*, 2018). Free-living or symbiotic cyanobacteria are classic diazotrophic organisms at early successional stages (Walker et al. 2003; Schmidt et al. 2008; Menge and Hedin 2009; Raggio et al. 2012; Arróniz-Crespo et al. 2014). Later in the succession, vascular plant colonization and tree establishment can bring endosymbiotic α-Proteobacteria (i.e. *Rhizobium*) or Actinobacteria (i.e. *Frankia* with *Alnus*) as more relevant N fixers (Lawrence *et al.*, 1967; Chapin *et al.*, 1994; Kohls *et al.*, 2003). Surprisingly, N-fixation rates by symbiotic associations with vascular plants during primary

succession is seldom reported and, therefore, possibly underestimated in high-latitude regions.

Similarly, little is known about the fate of fixed N and the main pathways driving N acquisition by non N-fixers during the succession. The establishment of mycorrhizae is a key element in N and P acquisition by plants (Johnson *et al.*, 2016), and it has been proposed as essential during ecological succession (Lambers *et al.*, 2008; Dickie *et al.*, 2013; Johnson *et al.*, 2016). However, this relationship has been seldom explored in glacier forelands. N discrimination ($\delta^{15}N$) values in plants are often used as an indicator of the occurrence of this process (Kohls *et al.*, 2003; Hobbie *et al.*, 2005). Mycorrhizal fungi can influence plant N economy and discrimination in several ways (see Hobbie and Högberg (2012) for detailed review). Mycorrhizae can not only increase plant access to recalcitrant and slowly diffusible forms of N and P but also, although the extent remains controversial, take up organic compounds and transfer them to their host (Näsholm *et al.*, 2008, 2013). Whilst their increased surface area and uptake capacities at low external concentrations may alter the average $\delta^{15}N$ of the available N sources, the latter are also markedly changed by the transfer to the host (Hobbie & Högberg, 2012). In particular, creation of transfer compounds by mycorrhizal fungi leads to retention of ^{15}N-enriched N and transfer of ^{15}N-depleted N to the plant symbiont. This ^{15}N fractionation probably occurs in all autotrophic mycorrhizal plants and normally it is difficult to directly link plant $\delta^{15}N$ to soil N sources because the exact $\delta^{15}N$ of the latter is rarely known. However, the glacier foreland in front of the Pia Glacier is unusual in that initial soil N contents are undetectable (Arróniz-Crespo *et al.*, 2014), atmospheric deposition is one of the lowest in the world (Dentener *et al.*, 2006), and there is almost immediate disconnection of exposed surfaces from the melt water from the glacier. In consequence, the vast majority of the N in the glacier foreland of the Pia Glacier comes potentially from N-fixation. This chronosequence thus provides the possibility to investigate both sources of N and the potential role of mycorrhizae in transferring N to non-fixing plants.

N availability directly impacts on primary productivity, which in turn rests on photosynthetic activity. Photosynthetic organisms in glacier forelands (i.e. photosynthetic bacteria, free-living and symbiotic algae, bryophytes, and vascular plants) are of paramount importance in exponentially increasing soil organic fraction along plant succession. Microbial and cryptogamic communities certainly help to ameliorate the initial harsh condition of barren exposed substrates but their impact on C stock is limited compared to

the contribution by vascular species (e.g. grasses or shrubs), which causes a substantial increment in soil organic content (Crocker & Major, 1955; Matthews, 1992; Chapin *et al.*, 1994; Arróniz-Crespo *et al.*, 2014). However, photosynthetic rates at glacier forelands have been mostly evaluated on cryptogams (Uchida *et al.*, 2006; Yoshitake *et al.*, 2010; De los Ríos *et al.*, 2011; Raggio *et al.*, 2012), and integrated approaches evaluating these species together with vascular plants are scarce (Muraoka *et al.*, 2002). Thus, improving our knowledge on photosynthesis during primary succession is not only essential to accurately characterize the major source of organic C at each successional stage but also to obtain some level of understanding of C-fixation limitations.

Ecological primary succession is not always straightforward (Walker *et al.*, 2010) and takes several decades and even hundreds of years (e.g. Viereck 1966; Reiners et al. 1971; Chapin et al. 1994; Sigler and Zeyer 2004; Jones and Del Moral 2005; Schmidt et al. 2008; Hodkinson et al. 2009). However, this pattern has been recently challenged by the fast succession occurring in front of the receding Pia Glacier (Tierra del Fuego, Chile; Sancho et al. 2011). Despite the lack of abiotic N inputs in this region (<0.7 kg N ha^{-1} yr^{-1}; Dentener et al. 2006), total soil N content reaches 1.5% (dry weight basis) after 34 years (Arróniz-Crespo *et al.*, 2014) and a *Nothofagus* dominated forest is in place with trees reaching 10 m in height (Sancho *et al.*, 2011). It appears that the trees show few signs of N limitation suggesting that another process is incorporating N into the ecosystems, which is almost certainly N fixation. The presence of free-living cyanobacteria and cyanobacteria associated with bryophytes and lichens has been reported but their N contribution to the succession is limited by its restriction to initial stages (Raggio *et al.*, 2012; Arróniz-Crespo *et al.*, 2014).

Gunnera magellanica Lam. is a native N-fixing herb which thrives profusely in Tierra del Fuego (Wanntorp & Wanntorp, 2003; Fernández-Martínez *et al.*, 2013). It is commonly found inhabiting glacier forelands (Henríquez & Lusk, 2005; Troncoso *et al.*, 2013; Pérez *et al.*, 2014), and belongs to the family Gunneraceae, the only angiosperm family known to establish endosymbiotic relationship with cyanobacteria from the genus *Nostoc* (Osborne and Bergman 2009; Santi et al. 2013). Although the uniqueness of the endosymbiotic relationship of *Gunnera* spp. has attracted scientific attention for decades (Silvester & Smith, 1969; Silvester & McNamara, 1976; Bergman, 2002; Bergman & Osborne, 2002; Osborne & Bergman, 2009), this genus has been surprisingly neglected when studying the role of N fixers in ecosystem succession, as well as in global estimations of biological N-fixation (Cleveland et al. 1999; Vitousek et al. 2013). *Gunnera magellanica* is present at almost all stages of the

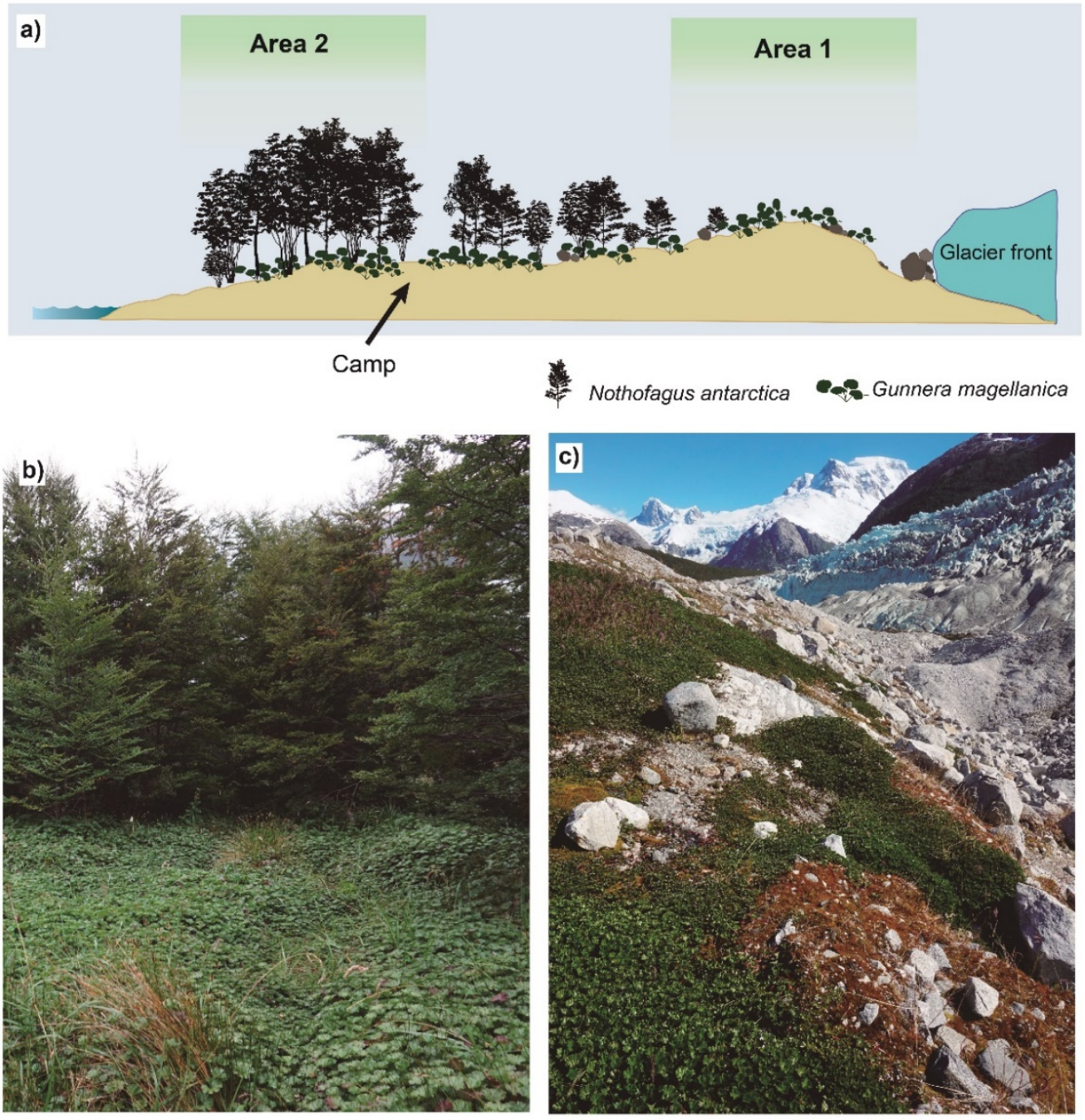
succession in Pia Glacier foreland, even under *Nothofagus* spp. forest canopy (Sancho *et al.*, 2011). It has been recently suggested that the N fixing capacity of this species may be high (Pérez *et al.*, 2017), putting *G. magellanica* as a good candidate to provide the considerable N input which must be required to drive the rapid primary succession observed in Tierra del Fuego.

Here, we measured the in situ N-fixing capacity of the flowering plant *G. magellanica* in front of the Pia Glacier (Tierra del Fuego, Chile) to determine if this species could be considered as a good candidate as a source of N in this area. We also measured the C fluxes (photosynthetic performance and respiration) in order to compare the performance of *G. magellanica* with other co-dominant species present in the succession. We determined foliar $\delta^{15}N$ and elemental contents of leaves of dominant species. We hypothesize that: (1) *G. magellanica* will show a considerably higher N-fixation rate compared to the other N-fixers in the same area (lichens and mosses). In other words, *G. magellanica* is the species providing the large quantity of N required to support the extraordinary fast succession at Pia Glacier; (2) the photosynthetic performance of *G. magellanica* will be promoted by its higher N availability due to the direct N supply from the endosymbiotic cyanobacteria; (3) the higher N availability and C acquisition capacity of *G. magellanica* will also drive higher P tissue contents in this species; (4) that mycorrhizae play key role in N acquisition and transfer.

Materials and Methods

Site description

The study site was located in the morainic field exposed after the retreat of two branches of Pia Glacier in Pia Bay, on the north side of the Beagle Channel in Tierra del Fuego, Chile (54°46'S 69°40'W). The climate of the area is dominated by a high precipitation (1600 mm) with mean annual air temperature of c. 4.5 °C (Sancho *et al.*, 2011). The deposited morainic ridges, consisting mainly of crystalline, granitic, or metamorphic materials, formed over 30-40 years (see Sancho et al. (2011) for detailed chronosequence description) have been rapidly colonized by different plant species with a well grown forest of *Nothofagus antarctica* (G. Forst.) Oerst. and *N. betuloides* (Mirb.) Oerst. on the older surfaces (Fig. 1b). The herbaceous layer on the ground is overwhelmingly dominated by *Gunnera magellanica*, which appears almost immediately after glacier retreat, within the first 4 years, and develops a dense cover (Fig. 1c; Table 1). The initial stages of succession are dominated by cryptogamic species, most



Fuego, Chile). Area 1 (moraine) and 2 (forest) represent the sampling locations and the arrow indicates the location of the field camp. (b) *Nothofagus* spp. forest (forest area) with dense cover of *Gunnera magellanica* at the herbaceous layer after 34-40 yr. of soil exposure. (c) Pia Glacier front (moraine area) and the initial colonization stages of *G. magellanica* after ~ 4 yr. of soil exposure.

of which establish cyanobacterial symbiosis. See Arróniz-Crespo et al. (2014) for a detailed description of moss and lichen communities in this area.

Table 1: Plants analyzed for leaf element and isotopic (^{15}N) analyses, indicating the life-form and type of mycorrhizal association. Species distribution and site age determination along the chronosequence are provided in Arróniz-Crespo et al. (2014). VAM: Vesicular-Arbuscular Mycorrhiza; Eric.: Ericoid mycorrhiza; Ecto.: Ectomycorrhiza.

			Presence (site age in years)				
Species	Form	Mycorrhizal type	4	7	10	19	34
Gunnera magellanica	Herb	VAM	+	+	+	+	+
Gaultheria mucronata	Shrub	Eric.		+	+	+	+
Nothofagus antarctica	Tree (deciduous)	Ecto.			+	+	+
Nothofagus betuloides	Tree (evergreen)	Ecto.		+	+	+	+

Sampling design

We visited the Pia Glacier foreland during the Austral summers of 2009 and 2015. Plant material (i.e. mature, sun-exposed leaves) was collected in 2009 from different species (Table 1) along the chronosequence in front of the Pia Glacier. Where possible, a total of 25 samples were collected for each species at each site (5 replicates at 5 sample points; see Green et al. (2017) for further description). In a second visit in 2015, we performed *in situ* measurements of C fluxes and N-fixation. To do this, we collected complete *Gunnera magellanica* plants from two moraine areas parallel to the glacier front (Fig. 1): area 1: immediately close to the glacier front and corresponding to ~4 yr. of soil exposure (hereafter called "moraine"), dominated by cryptogamic species (mainly *Placopsis* spp., *Peltigera patagonica* and various mosses; see Arróniz-Crespo et al. 2014) but with initial *G. magellanica* colonization; and area 2: c. 200 m distant from glacier front, with a well-developed forest of *Nothofagus* spp. after ~34-40 yr. of soil exposure (hereafter called "forest"), with *G. magellanica* dominating the herbaceous layer and occasional cryptogams growing on rocks in forest clearings. At both areas, six sampling plots (0.5 x 0.5 m) were randomly selected with at least a 10 m separation to ensure independence between plants (*G. magellanica* tend to reproduce by stolon production). At each plot, two samples consisting of soil sections (10x10x10 cm) containing *G. magellanica* plants (including roots), were collected and transported to our base camp in forest area (Fig. 1ab). One sample was used for photosynthetic measurements and the other

for N-fixation analysis (plants were processed differently, see description below). Controls for the effect of transplanting on plant photosynthetic performance were included, and consisted of similar soil sections with *G. magellanica* plants (n = 5) naturally growing in forest area close to the base camp (and within the measurement range of the equipment). Important N-fixing lichen species were also sampled: *Placopsis perrugosa* and *Peltigera patagonica* (lichens only present in moraine area), and the lichen *Stereocaulon alpinum* (present only in forest area). Lichen thalli were collected with a similar spatial replication as described for *G. magellanica* and transported to base camp. The tree *N. antarctica* was also used for C-fixation measurements in forest area with measurements made on exposed leaves of a well-developed tree growing near the base camp.

Measurements of carbon fluxes

Carbon fluxes (CO_2-exchange - photosynthesis and respiration) were measured under field conditions using a portable open flow IRGA system (GFS 3000, Walz, Germany). Measurements were made over four days and nights for *G. magellanica* and *N. antarctica* samples, and three days and nights for lichens. The measuring period covered a wide range of climatic conditions which are probably representative of the study area during the growing season. During C flux measurements, incident photosynthetic active radiation ($\mu mol \cdot m^{-2} \cdot s^{-1}$, PAR), temperature inside the measuring cuvette (^oC; Tcuv) and relative humidity (rh) were recorded as these can all influence C fluxes. Leaves (n = 5) of the *N. antarctica* tree were measured at the same time as *G. magellanica* (n = 5). All samples were measured at intervals through each day. Similarly, C fluxes of lichens (n = 5 for each species) were measured over 3 days. The first day was a dry day, meaning that lichens were inactive and were artificially wetted in the evening by spraying with water taken from the glacier. On the remaining two days, measurements were done under natural conditions because the lichens had been hydrated by rain.

Leaf element analysis

Leaf N and P analysis was performed by ICP/MS (inductively coupled plasma mass spectrometry) at Waikato University, Hamilton, New Zealand as described in Green et al. (2017). Leaf ^{15}N contents were measured by the Waikato Stable Isotope Unit (WSIU, Waikato University, Hamilton, New Zealand) using their internationally accredited methods.

Mycorrhizae and ^{15}N discrimination – theory

In an open system, the proportion of N taken up by mycorrhizal fungi that is then passed on to host plants, termed the transfer ratio (Tr), can explain N isotope patterns in mycorrhizal plants according to the following equation (Hobbie *et al.*, 2000a):

$$\delta^{15}N_{plant} = \delta^{15}N_{available\ nitrogen} - \Delta f \times (1 - T_r) \times f$$

where Tr is the transfer ratio (between 0 and 1), Δf is the proportion of the N in the plant that comes from the fungus, and f is the discrimination against ^{15}N during the creation of transfer compounds of 8–10‰.

When mycorrhizae are involved in transferring fixed N to their host plants, large changes in $\delta^{15}N$ can occur. The extent of these changes is influenced by the type of mycorrhizal association formed by plants (Hobbie & Högberg, 2012), with foliar $\delta^{15}N$ decreasing in the order non-mycorrhizal (mean ± SE, 0.9 ± 0.2‰) > arbuscular mycorrhizal (-1.1 ± 0.1‰) > ectomycorrhizal (-2.3 ± 0.2‰) > ericoid mycorrhizal plants (-5.0 ± 0.2‰). Non-mycorrhizal plants are enriched in ^{15}N relative to all mycorrhizal types. The transfer of ^{15}N depleted N from fungi to plants coupled with N retention by fungi leads to ^{15}N-depleted plants and ^{15}N-enriched fungi so that the ^{15}N patterns can provide insight into N partitioning between mycorrhizal fungi and host plants.

Biomass and apex density estimates

Gunnera magellanica plants spread by rhizomes, so plant density (plants · m^{-2}) was determined as the number of rhizome apices in each 10x10 cm sample. The projected area of the lichens *P. patagonica P. perrugosa* and *S. alpinum* samples was determined from photographs using ImageJ software version 1.50i. Dry weight was obtained for *G. magellanica, P. patagonica* and *S. alpinum* by drying in an oven at 60 ºC for 72 h., and for *P. perrugosa* (lichen is firmly attached to the rock) by change in weight after burning the samples at 850 ºC for two hours.

Nitrogen fixation analysis

Nitrogenase activity was estimated in parallel to C fluxes measurements, under field conditions, by using the acetylene reduction assay (Hardy et al. 1968). *Gunnera magellanica* samples (n = 5 for each sampled area) were enclosed in 1 L glass incubation bottles fitted with a rubber septum. Lichen samples (*P. patagonica, S. alpinum* and stones holding *P. perrugosa* thalli) were enclosed in wide-mouth glass jars of 125 mL with PTFE-faced silicone septum.

Prior to incubation, soil and debris attached to *G. magellanica* roots or lichen thalli were eliminated and kept hydrated by spraying. Incubation bottles were placed under *Nothofagus* canopy to avoid warming by direct sun exposure, but with enough light to ensure active photosynthesis. Temperature inside and outside of bottles was continuously monitored with iButton® (Fig. S1). For each species, and for each studied area in case of *G. magellanica*, one sample was incubated without acetylene as a control to detect basal ethylene production. The other samples (n = 5 for each species) were incubated with acetylene produced *in situ* by adding water to calcium carbide, in a carbide lamp and stored in Tedlar® gas sampling bags until extracted for injection. 10 % of the bottle volume was replaced by acetylene resulting in headspace enrichments of ~ 10 % (enrichment was determined individually for each species allowing for the different vial headspaces, see Appendix S1). After 1 hour incubation, a gas sample was extracted using a gastight syringe and stored in 5.7 mL Exetainer® evacuated gas sampling vials. Samples were aerated between incubations to prevent long-term incubation effects on nitrogenase activity (Dart & Day, 1971; David & Fay, 1977; Wani *et al.*, 1983). This process was repeated, with the same samples, at 3 hour intervals over a complete 24-hour cycle. Plants and lichen thalli were kept hydrated between incubations by covering roots and rhizomes with wet tissue or spraying thalli. Vials were transported to Madrid for ethylene concentration determination by GC: Varian 3300 gas chromatograph equipped with a J&W Agilent HP-PLOT Q column and a flame ionization detector. The N-fixation rates were then calculated from the ethylene concentration assuming the mostly accepted C_2H_4:N conversion factor of 3:1. N fixation was then expressed as nmols $C_2H_4 \cdot g\ DW^{-1} \cdot h^{-1}$ and $\mu g\ N \cdot g\ DW^{-1} \cdot d^{-1}$. An estimate of annual species N contribution to the ecosystem was calculated from nitrogenase activity assuming 100% cover and continuous activity and expressed as $kg\ N \cdot ha^{-1} \cdot yr^{-1}$.

Statistical analysis

Because measurements of both N-fixation and C fluxes were performed by repeatedly sampling the same individuals at specific intervals, samples were not independent of each other; we thus performed a repeated-measure approach. To assess the significant differences of the N-fixation rates of *G. magellanica* plants between different habitats we conducted a two-way PERMANOVA analysis (Anderson, 2001), based on Euclidean similarity matrix with habitat (two levels: moraine and forest) treated as fixed factor, and time (seven levels) and plant replicate at each habitat (five levels, nested within habitat) treated as random factors. Differences in the N-fixation rates of the three lichen species studied (*P. patagonica*,

P. perrugosa and *S. alpinum*) were tested by a similar approach, but without considering habitat and with three levels in the species factor. We followed a more complex approach to test the differences between the photosynthetic performance and respiration of *G. magellanica* and *N. antarctica*. We assessed the effect of treatment on photosynthetic performance of *G. magellanica* as well as differences between *G. magellanica* and *N. antarctica* by conducting a four-way PERMANOVA analysis, with species (two levels) and treatment (three levels: control, transplanted forest and transplanted moraine, nested within species) as fixed factors, and time (12 levels) and plant replicate (five levels, nested within treatment) treated as random factors. PAR, Tcuv and rh were included as covariates of photosynthesis to account for differences in the measuring conditions between samples. We then followed a similar approach to test the photosynthetic performance and respiration of the two lichens (*P. patagonica* and *P. perrugosa*) measured. *Stereocaulon alpinum* was initially considered but finally discarded from C fluxes measurements because of the low rates measured for this species. We used a two-way PERMANOVA analysis, with species (two levels) as a fixed factor, and time (11 levels) and lichen species replicate (five levels, nested within species) treated as random factors. Similarly, PAR, Tcuv and rh were included as covariates. PERMANOVA analyses were developed using 9999 permutations (permutation of raw data) with the PERMANOVA+ for PRIMER v6 statistical package (PRIMER-E Ltd., Plymouth Marine Laboratory, Ivybridge, UK). Differences between habitats (in case of N-fixation), and between treatment and species (in photosynthesis and respiration assessments) were tested by using pairwise post hoc tests in PERMANOVA

Finally, to help the interpretation of the influence of morphology on N-fixation rates of *G. magellanica* from both localities, we evaluated the differences in biomass and plant density between *Gunnera magellanica* plants from localities by conducting one-way ANOVA.

Results

Nitrogen fixation rates

Nitrogenase (acetylene reduction) activity showed similar, and marked, diel variation of nitrogenase activity for all studied species, with the highest values around midday (12-15 p.m.) and lowest values before sunrise (4-5 a.m.; Fig. 2). *Gunnera magellanica* plants from the moraine and forest had the highest mean values of nitrogenase activity both per dry weight and area basis, respectively (Fig. 2, Table 2), but only differed differed significantly on a dry weight basis (P = 0.041; Table S1). The lichen species *P. perrugosa* and *P. patagonica* had

Table 2: Nitrogen fixation rates (averaged) for evaluated species from both sampling locations obtained from day cycles of nitrogenase activity measured by acetylene reduction assay. In case of *Gunnera magellanica*, average estimates using all data from both sample sets is provided as *overall*. Data are means ± SE (n = 5). Estimated rates were calculated assuming 100% coverture of selected species and, in case of lichens, assuming continuous nitrogenase activity (as poikilohydric organisms they will be inactive at dry periods).

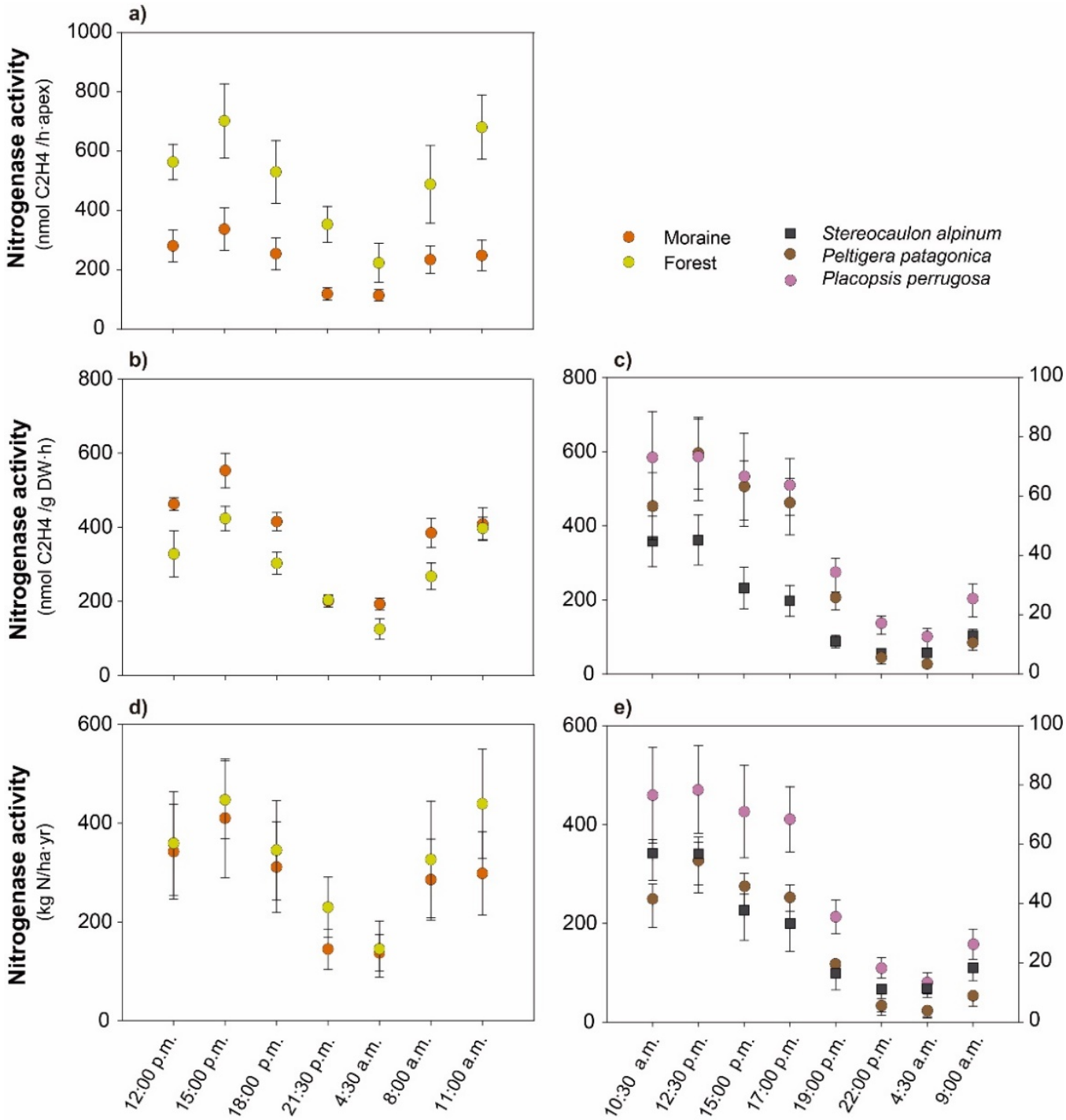
Species	*G. magellanica*			*P. patagonica*	*P perrugosa*	*S. alpinum*
Location	Moraine	Forest	Overall	Moraine	Moraine	Forest
Measured rates						
nmols C_2H_4 / g DW·h	373.32 (±50.18)	292.39 (±39.68)	341.76 (±15.82)	290.28 (±6.72)	359.61 (±34.06)	22.74 (±2.48)
nmol C_2H_4 / cm^2·h	67.41 (±9.3)	80.1 (±10.05)	74. 15 (±5.7)	37.13 (±10.6)	11.84 (±2.4)	6.81 (±1.65)
Estimated rates						
µg N / g DW·d	41.86 (±5.62)	32.75 (±4.44)	38.28 (±1.77)	32.51 (±9.25)	40.28 (±8.17)	2.55 (±0.63)
g N / m^2·yr	27.56 (±3.8)	32.73 (±4.11)	30.31 (±2.33)	15.18 (±4.33)	4.84 (±0.97)	2.78 (±0.67)
kg N / ha·yr	275.58 (±38)	327.33 (±41.08)	303.11 (±23.3)	151.8 (±43.32)	48.41 (±9.8)	27.84 (±6.72)

similar nitrogenase activities on a dry weight basis (P = 0.561; Table S1), and also close to those obtained for *G. magellanica* (Table 2). The lichen *S. alpinum* had the lowest nitrogenase activity, on a dry weight and area basis and was significantly lower than *P. perrugosa* and *P. patagonica* (Table 2, S1, $P < 0.05$). All lichen species were significantly different when nitrogenase activity was expressed on an area basis ($P < 0.05$; Table S1), with *P. patagonica* having the highest rate (Table 2).

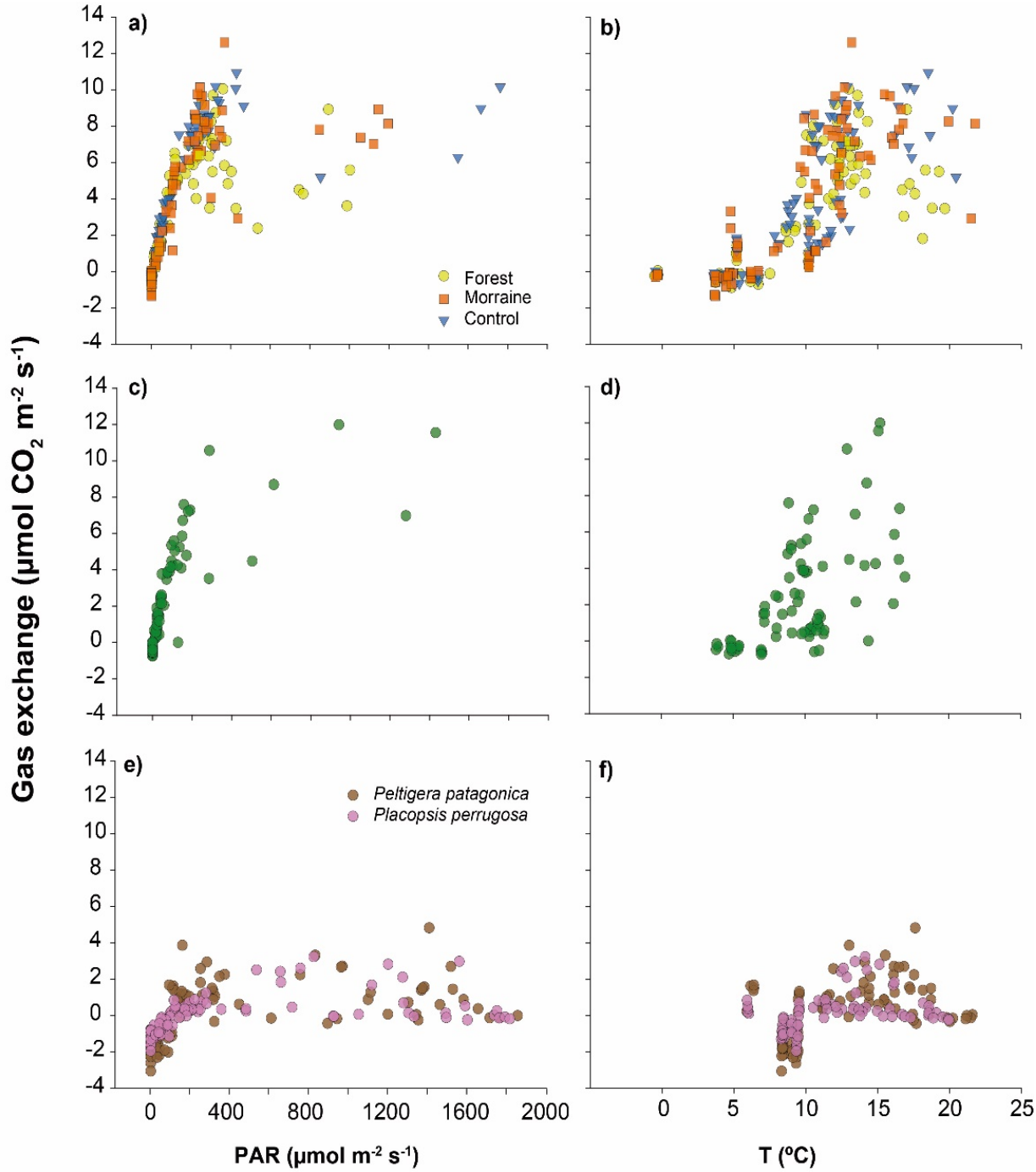
The estimated N contribution of *G. magellanica* to the succession showed no statistical difference between areas (P = 0.996), with 276 and 327 kg N $ha^{-1}{\cdot}yr^{-1}$ for plants from area 1 and 2, respectively (Table 2). Regarding lichen species, the estimated N contribution was significantly higher for *P. patagonica* (151.8kg N $ha^{-1}{\cdot}yr^{-1}$; $P < 0.005$) than the observed for *P. perrugosa* and *S. alpinum* (48.41 and 27.84 kg N $ha^{-1}{\cdot}yr^{-1}$ respectively). The calculated daily and annual contributions need to be treated with caution as they assume 100% activity and cover. The actual amounts will be lower for *G. magellanica* because of colder temperatures and probable leaf loss in winter, and much lower for the lichens because they are poikilohydric and inactive when dry.

Comparison of carbon fluxes between locations and species

Our results showed no statistical effect of transplant on photosynthetic performance of *G. magellanica* plants (Table S2). Net photosynthesis (area basis) was significantly higher for *G. magellanica* in the forest (area 2; P = 0.005; Table S2). Net photosynthetic rates were highly significantly higher for the herb *G. magellanica* compared to the he tree species *N. antarctica* (P = 0.005; Fig. 3). In contrast, both species showed a very similar saturation pattern in their response to light (Fig 3 ab). Regarding respiration, we did not find significant differences between *G. magellanica* pairs (moraine and forest) nor between that species and *N. antarctica* (Table S2). The lichens *Peltigera patagonica* and *Placopsis perrugosa* had much lower net photosynthetic rates than for both *G. magellanica* and *N. antarctica* but a similar pattern of response to ambient light (Fig. 3 c). All species showed similar response of CO_2 exchange rates to temperature (Fig 3 d,e,f), with an optimum temperature around 15 ºC. However, whereas *G. magellanica* had a relatively clear optimum around 15 ºC, the patterns for *N. antarctica* and the lichen species were less clear.



considered: *Gunnera magellanica* (a,c;d; ●: *G. magellanica* moraine; ●: *G. magellanica* forest) and N fixing lichens (c and e; ■: *Stereocaulon alpinum*; ●: *Petigera patagonica;* ●: *Placopsis perrugosa*). Data are means (n = 5) ± SE. Note that *Stereocaulon alpinum* (graphs c and e) and *Placopsis perrugosa* (graph e) refer to the secondary axis on the right.



natural conditions of light (a,b,c) and temperature (d,e,f) for different species considered: *Gunnera magellanica* (a and d; square: *Gunnera* moraine; circle: *Gunnera* forest; inverted triangle: *Gunnera* control), *Nothofagus antarctica* (b and e) and N fixing lichens (c and f: dark square: *Placopsis perrugosa*; open square: *Petigera patagonica*). N= 5 for all cases except for *Nothofagus* tree, where 5 leaves of same individual were used. PAR: photosynthetic active radiation: T: temperature.

Leaf N and P contents

G. magellanica leaves had a consistently higher N and P content compared to the rest of analyzed plant species (Table 3; Fig. 4 a,b). *Gunnera magellanica* leaves contained three times more N than the shrub species *G. mucronata*, twice that of the tree *N. betuloides* and 30 % higher than the tree *N. antarctica* (Table 3, Fig. 4a). The seral shrub *G. mucronata* had the lowest N contents, ~1%, whilst the deciduous *N. antarctica* had N contents around 2.2 – 3.0% and the evergreen *N. betuloides* a much lower ~1.5%. The results are very similar for leaf P content with little indication, if little, of P deficiency and with P leaf contents of each species remaining almost constant across all sites (except for *N. betuloides* at early stage; Fig. 4b). However, there were species-specific difference in P content with the same rank order as for N content (*Gaultheria* lowest, then *N. betuloides*, *N. antarctica* and *Gunnera* highest).

Table 3: Averaged values of foliar N (%), P (ppm) and $\delta^{15}N$ obtained from the evaluated species at forefront and mature sites. Data are means (n = 25) ± standard error.

Species	N	P	N:P	$\delta^{15}N$(‰)
Gunnera magellanica	3.253 (0.0933)	2898.66 (236.21)	11.44	-0.31
Gaultheria mucronata	1.041 (0.034)	840.19 (60.11)	11.97	-4.82
Nothofagus betuloides	1.390 (0.102)	1779.10 (471.48)	11.95	-4.03
Nothofagus antarctica	2.451 (0.273)	1913.12 (40.70)	12.65	-3.85

Leaf stable isotope values

Foliar $\delta^{15}N$ results indicated that the plants along the chronosequence can be divided into two groups based on the absolute values of $\delta^{15}N$ and changes with time (Fig. 4c). The first group contains only *G. magellanica* with $\delta^{15}N$ close to zero and little change with increasing age of the substrate. The second group contains the three species *N. antarctica, N. betuloides* and *G. mucronata* which always had negative $\delta^{15}N$. Discrimination values of these species were initially low and then rose to be least negative at 10 years (-2.0625‰, -2.5071‰, and -3.3837‰, respectively). $\delta^{15}N$ values then became increasingly negative at older sites with a steady and very similar decline to around -6‰ after 34 years. In contrast to their N-contents all three species have very similar $\delta^{15}N$ at the older sites, despite possessing different mycorrhizal types (Table 1). All species present on surfaces younger than 10 years show a negative $\delta^{15}N$ including *G. magellanica* (Fig. 4c).

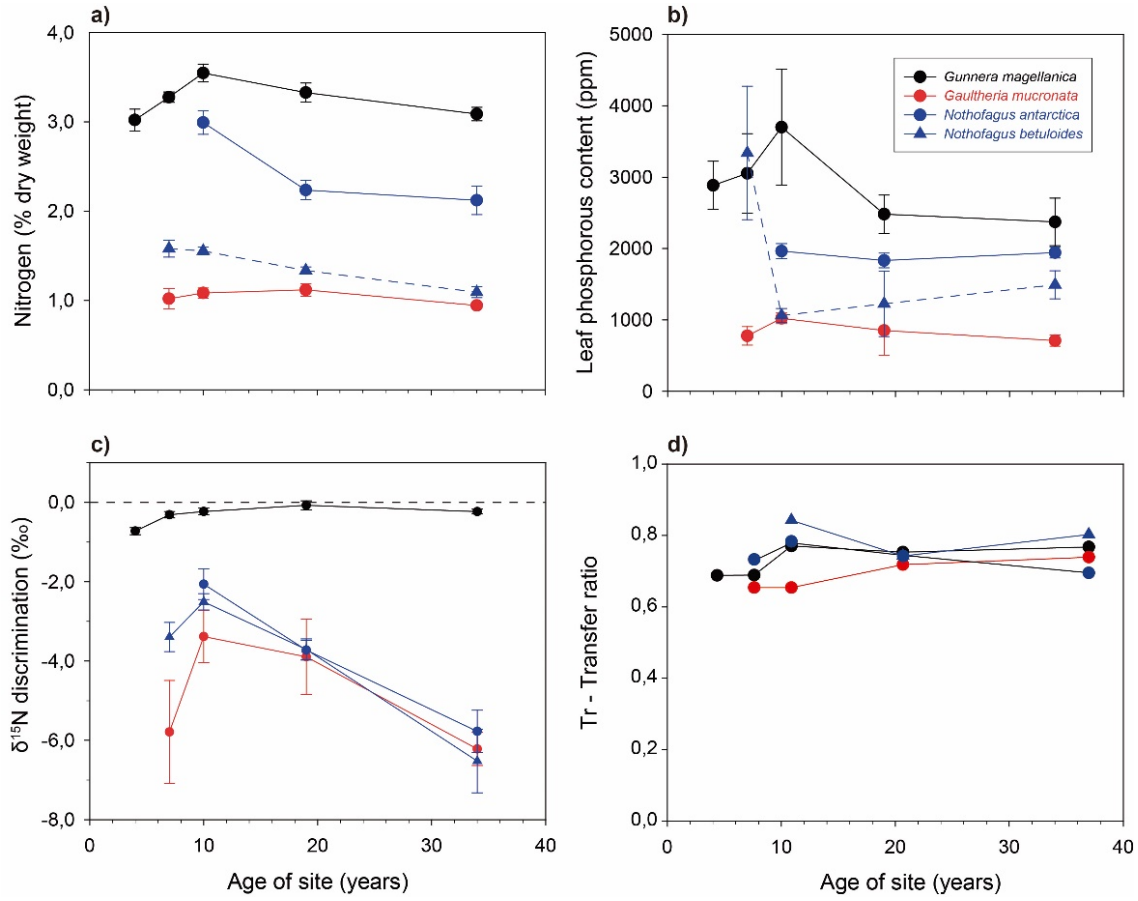


Figure 4. Leaf elemental and isotopic analysis in selected plant species after glacial retreat at Pia Glacier, Tierra del Fuego (Chile) indicated in Table 1. a) Leaf N content (% DW); b) Leaf P content (ppm); c) $\delta^{15}N$ patterns reflecting development of mycorrhizae and nitrogen dynamics during primary succession; d) estimated values for Tr (transfer ratio for N from fungus to host) calculated for each specie, and along the chronosequence, making the following assumptions: F is set at 0.7 for all plants except *G. magellanica* which gets its nitrogen from the air and has been set at 0.1. Non-mycorrhizal plants receive N from their mycorrhizae calculated as 90% sourced from *G. magellanica* at 7 years, 70% at 10 years, 50% at 19 years and 30% at 34 years (mean $\delta^{15}N$ = -0.74, -0.96, -1.92 and -4.39 ‰, respectively). $\delta^{15}N$ available to *G. magellanica* has been adjusted in the early years to reflect that it is coming mainly from the melt water from the glacier. Values are mean (n = 25) ± SE.

Discussion

Our study provides evidence that N inputs via N-fixation can explain the rapid primary succession in the Pia Glacier foreland (Tierra del Fuego, Chile) and that plant-microbial symbiosis potentially supports this succession through N-fixation (*G. magellanica* and cyanobacteria), N transfer (mycorrhizae) and possibly improved P uptake (mycorrhizae). Answering our initial hypothesis, we found that: (1) the perennial herb *G. magellanica*

appears to be the most important N-fixing species evaluated in the area with the highest N-fixation activity yet reported within the family Gunneraceae and an average N-fixation rate of 342 nmols C_2H_4·g DW^{-1}·h^{-1}, which equates a potential N contribution to the ecosystem of around 300 kg N·ha^{-1}·yr^{-1}. This large N contribution is not only the result of its intrinsic high nitrogenase activity but also because of its high cover throughout the succession. (2) The photosynthetic performance of *G. magellanica* is slightly, but significantly, better than that of *N. antarctica* although all the species showed rates similar to those commonly observed for similar plants in other regions worldwide. (3) The $\delta^{15}N$ discrimination values suggests that N is rapidly acquired by shrub and tree vegetation via mycorrhizae. (4) *Gunnera magellanica* does have higher P contents than the other species and that P content shows the same rank order as leaf N content. Overall, extraordinarily high N-fixation and efficient, and potential fast pathways for nutrient turnover are driving this succession despite low natural abundances of N and P and these results highlight the uniqueness of the ecological conditions at Pía Glacier.

Nitrogen fixation rates

The comparison of the values of nitrogenase activity obtained for *G. magellanica* with previously reported rates for other N-fixing plants must be done carefully. Several problems arise when comparing with existing data, as the units used to express N-fixation rates in plants are not consistent in the literature (e.g. rates expressed in terms of fresh weight or mg of cyanobacterial pigment/protein). Similarly, the comparison with nodulated species is also difficult, as most studies express the rates referred only to nodule dry weight (Uliassi & Ruess, 2002). Our results for *G. magellanica* N-fixation activity are higher than those reported for pioneer plants in primary successions (Blundon & Dale, 1990) or lower in terms of plant material (as an artifact of using only nodule biomass) but much higher in area basis (Lepper & Fleschner, 1977; Vitousek & Walker, 1989; Halvorson *et al.*, 1992). However, there is a surprising gap in our knowledge of N fixation rates of N-fixing plants in primary succession in glacier forelands, limiting further characterization of our results. In addition, the rates of *G. magellanica* generally exceed the rates obtained or reported for other lichen and moss species in symbiosis with cyanobacteria across the same chronosequence (Raggio *et al.*, 2012; Arróniz-Crespo *et al.*, 2014). However, the cephalodiated lichen *Placopsis perrugosa* showed similar averaged daily nitrogenase activity (dry weight basis; nmols $C_2H_4 \cdot g^{-1}$ DW·h^{-1}) than *G. magellanica* (Table 2). This result is certainly surprising, but the poikilohydric nature of this lichen and its low coverture compared with the N-fixing herb, prevents considering this

estimation a comparable figure. Moreover, its high rate may be explained by the thin thallus of this lichen species and its low dry weight per unit area (Raggio *et al.*, 2012). Nevertheless, our results for *P. perrugosa* are similar to those previously reported for other *Placopsis* spp. growing on Pia Glacier moraine (Raggio et al. 2012) or other regions (Crittenden, 1975). The highest fixation per unit area basis was observed for the foliose lichen *Peltigera patagonica*, a native species restricted to Patagonia. The low activity observed for the cosmopolitan fruticose species *Stereocaulon alpinum*, member of a widespread pioneer genus in primary succession (Vitousek, 1994; Kurina & Vitousek, 1999), is congruent with literature, as this genus is generally linked to low N contributions (Kurina & Vitousek, 2001). All studied species, except the lichen *S. alpinum*, showed higher N-fixation rates than those reported from moss species across the same glacier foreland (Arróniz-Crespo *et al.*, 2014), but the moss *Ditrichum cylindricarpum*, with an averaged N-fixation rate of 283.4 nmol $C_2H_4 \cdot g^{-1}$ $DW \cdot h^{-1}$, was comparable to the evaluated species *G. magellanica*, *P. patagonica* and *P. perrugosa*. Thus, a high nitrogenase activity seems to be common in different organisms at Pia Glacier foreland.

Diel variation of nitrogenase activity is an often reported trend in N-fixation estimations (Balandreau *et al.*, 1974; Halvorson *et al.*, 1992; Lee & Son, 2005), related to the tight dependence of this enzyme to temperature, water availability and –indirectly via fixed C allocation– light (Waughman, 1977; Gibson & Jordan, 1983). In case of *G. magellanica*, temperature is the most likely factor driving the observed diel variation in nitrogenase activity, as leaf removal did not result in a short-term reduction of N-fixation capacity in *G. magellanica* shoots (data not shown). This is in accordance with previous studies showing that temperature, and not light, primarily controls daily variation in root nodule activity (Eckart & Raguse, 1980; Schweitzer & Harper, 1980).

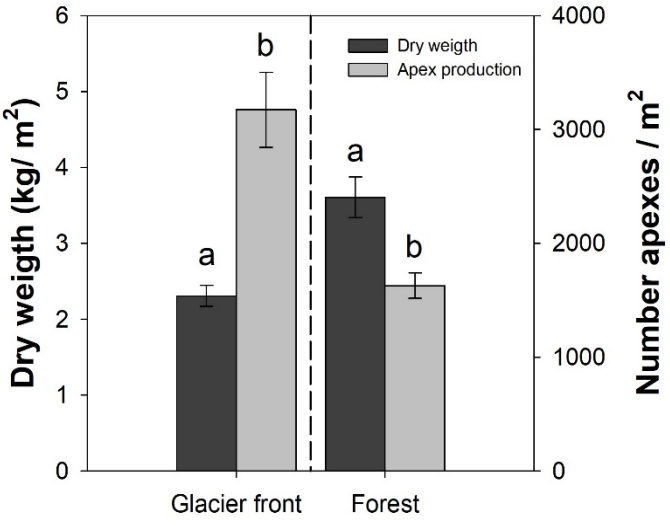


Figure 5. Biomass and apex production of *Gunnera magellanica* in the contrasting locations sampled: glacier front and forest. Bars represent means ± standard error (n = 10). Different letters represent statistical differences (P < 0.005, ANOVA).

Thus, we expect a reduction of N-fixation activity of *G. magellanica* by low temperature during austral winter season, although oceanic climate conditions of the studied area prevent extreme temperature oscillations both daily and seasonal (Santana *et al.*, 2006).

With an averaged N contribution of 303.11 Kg N · m^{-2}·yr^{-1} to the ecosystem, *G. magellanica* largely exceeds that reported for other species of this genus, such as *G. arenaria* (72 kg N ha^{-1} yr^{-1}; Silvester and Smith 1969), or *G. macrophylla* (12-21 kg N ha^{-1} yr^{-1}; Becking 1976). However, we acknowledge that N-fixation rates of most of the Gunneraceae species are unknown. Further research is needed to successfully characterize our data. However, we observed a lower N contribution in the moraine area, at the initial stages of plant colonization, which may reflect a different plant reproduction and C allocation strategy (Fig. 5). The high N contribution found for *G. magellanica* at Pia Glacier is among the highest reported from nodulated phanerogam species (e.g. *Alnus*, *Casuarina*, *Acacia* and *Glycine*) and possibly only exceeded by a similar cyanobacterial symbiosis in the fern *Azolla* (Kellar & Goldman, 1979; Silvester, 1983; George *et al.*, 1988; Rennie *et al.*, 1988; Binkley *et al.*, 1994; Vaishampayan *et al.*, 2001). Our results contrast with previous attempts to provide tentative N-fixation rates of *G. magellanica* in Tierra del Fuego, which did not accurately detect its real scale. For example, estimations under laboratory conditions have reported lower N contribution (e.g. 10 kg N·ha^{-1}·yr^{-1}; Troncoso et al. 2013; Pérez et al. 2014) and *in situ* estimations of just 30 kg N·ha^{-1}·yr^{-1} (Perez et al. 2017). These discrepancies with our results may be explained by a reduction of the nitrogenase efficiency under laboratory conditions due to plant manipulation (Söderbäck *et al.*, 1990) in case of *ex situ* estimations, or different methodological approaches at *in situ* measurements (e.g. long incubation periods are known to affect the nitrogenase activity by multiple factors, Dart and Day 1971; David and Fay 1977; Wani et al. 1983). In addition, the consideration of other N fixing species with lower nitrogenase activities in estimates regarding *G. magellanica* might have masked its real nitrogenase activity (Pérez *et al.*, 2014). *Gunnera magellanica* has also been reported from other Fuegian glacier forelands with much slower successions (Arróniz-Crespo *et al.*, 2014). This discrepancy may be explained by the marked precipitation gradient that characterizes this region (decreasing from approximately 2000 mm at west coast to 200 mm at east parts; Tuhkanen 1992; Santana et al. 2006), with lower fixation and succession rates reported from drier regions (e.g. 800 mm at Parry Glacier; Arróniz-Crespo et al. 2014). Thus, more efforts are needed to accurately assess the N-fixation capacity of the phanerogam species *G.*

magellanica, exploring how different precipitation regimes affect the nitrogenase activity of this species.

The high nitrogenase activity and high N contribution of evaluated species detected in Pia Glacier foreland confirm the high N input suggested by Arróniz-Crespo et al. (2014) to support the high rates of biomass productivity in this extraordinarily fast primary succession (Sancho et al. 2011). Our results clearly contrast with previous N-fixation rates reported for glacier forelands (Crittenden, 1975; Blundon & Dale, 1990; Menge & Hedin, 2009), usually located in high-latitude/high-altitude regions (e.g. alpine or polar regions) where climatic conditions (i.e. low temperature) directly limit biological processes associated with N-fixation (Cleveland et al. 1999). For example, N-fixation rates reported from these habitats are usually very low, ranging from 0.4 to 5.0 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Cleveland et al. 1999; Reed et al. 2011). The deciduous nature of *G. magellanica* makes reasonable to expect a high N release by this species to the succession, and therefore a high N contribution to soil N, as occurs with other N-fixing shrubs in primary succession (e.g. *Coriaria* or *Alnus*; Crocker and Major 1955; Walker et al. 2003). However, the slower colonization rates and lower N contributions reported from other primary successions (Menge & Hedin, 2009) suggest that N contribution of *G. magellanica* is exceptional. Nevertheless, we acknowledge that the extrapolations of short-term estimates to annual rates are not realistic (Crews *et al.*, 2001) and our data only represent the maximum fixation rates of studied species in natural conditions (i.e. N-fixation during the most favorable season).

Nutrient content

Our results show that *G. magellanica* has a higher nutrient content (both N and P) than the other analyzed species in the same succession. These results are in agreement with the general pattern observed for N fixing plants, consistently reporting high N contents in plant tissue (Wright *et al.*, 2004; Nasto *et al.*, 2014; Adams *et al.*, 2016; Dong *et al.*, 2017; Guo *et al.*, 2017). However, we found larger N enrichment (between 30-300 %) compared to previous studies focused on legumes (Adams *et al.*, 2016; Guo *et al.*, 2017), with the highest difference observed between the pair *G. magellanica* – *G. mucronata*. It has been suggested that direct N supply by biological N fixation provides a competitive advantage to N fixing plants compared to their direct competitors lacking this trait (Vitousek & Howarth, 1991). This is, N fixers could invest more N in the photosynthetic complex (i.e. Rubisco) which in turn allows higher investment of N and C in the synthesis of phosphatases (Houlton *et al.*, 2008). This would explain why *G. magellanica* showed also the highest tissue P content as well as photosynthetic

performance (see below). Moreover, the lowest N:P ratio found for *G. magellanica* is in agreement with a higher maximum relative growth rate and biomass production (Güsewell, 2004) compared with the other studied plants, supporting the role of this species sustaining the fast plant colonization in the area. At the same time, the values observed for N:P ratios in the four studied plant species (between 10 and 20; Güsewell 2004) indicate a lack of N and P deficit for plant growth in the area.

N transfer processes

To achieve the rapid forest succession in Pia Glacier there must be a transfer mechanism that moves the fixed N to the non-fixing plants; as the latter all have high and clearly non-limiting N-contents (Fig. 4a), this transfer process must be also highly efficient. The key transfer process appears to be via mycorrhizae, as suggested by the negative $\delta^{15}N$ in plant tissues. Many studies have reported that ectomycorrhizal and ericoid mycorrhizal plants in Arctic, alpine or boreal regions were significantly depleted in ^{15}N relative to co-occurring arbuscular mycorrhizal plants (Schulze *et al.*, 1994; Michelsen *et al.*, 1996; Hobbie *et al.*, 2005). A major problem in interpreting foliar $\delta^{15}N$ is that multiple N sources can distort isotopic signatures. However, in the Pia Glacier foreland N supply is probably entirely from N-fixation and the $\delta^{15}N$ values of plants that rely exclusively on N-fixation are usually ~0 ‰ (as occurs for *G. magellanica*), reflecting atmospheric isotopic N values (Handley & Scrimgeour, 1997; Hobbie *et al.*, 2005). The application of the formula linking source $\delta^{15}N$ and plant $\delta^{15}N$ to the chronosequence using assumptions as the proportion of the N coming from *G. magellanica* (declines as recycling increases) produce a constant Tr at around 0.8 (Fig. 4d). This indicates that about 80% of N taken up by the fungi would be transferred to the mycorrhizal host. This is a high proportion that probably reflects the quantities of N being released from the N fixers but is also highly energetically efficient in terms of carbon supplied by the host (Hobbie and Högberg 2012).

C fluxes along the succession

The measurement of C fluxes, both photosynthetic activity and respiration, of *G. magellanica* showed a similar range than that observed for *N. antarctica,* although we found significant differences between both species, with higher photosynthetic values recorded for *G. magellanica*. As initially hypothesized, higher N content matched higher photosynthetic capacity, which has been profusely documented (Evans, 1989; Reich *et al.*, 1997; Niinemets, 1999; Farquhar & Miller, 2002; Wright *et al.*, 2004; Shipley *et al.*, 2005) but the

photosynthetic advantage derived from higher N content was much weaker than initially expected. In fact, the photosynthetic rates obtained for both herbaceous and tree species in Pia Glacier were scantly different, although in the same range previously reported from polar or temperate regions (Larcher, 1995). For example, similar net photosynthesis values have been reported from arctic dwarf shrubs or grasses (Muraoka *et al.*, 2008; Albert *et al.*, 2011), with similar saturation pattern around 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Moreover, the obtained photosynthetic values for *N. antarctica* are similar to previously reported for other *Nothofagus* spp. in this region (Martínez Pastur *et al.*, 2007). The slight differences in photosynthetic activities between N-fixers and non-N-fixers might be a consequence of the absence of N-limiting conditions (by both high N inputs and the efficient acquisition strategy in the system), minimizing the physiological advantage of N-fixers in this habitat.

The growth rate of both *N. antarctica* and *N. betuloides* in Pia Glacier (30 cm $year^{-1}$; Sancho et al. 2011) is similar to the previously reported growth rates for *Nothofagus* spp. (Cárdenas Garrido & Lusk, 2002; Salas & García, 2006) and exceed those reported for *Nothofagus menziesii* and *N. fusca* in New Zealand (Runkle *et al.*, 1997). This suggests efficient C allocation to aboveground biomass in *N. antarctica* during succession. *Gunnera magellanica* plants from forest and moraine showed divergent morphological adaptations (e.g. leaf size) and growing forms, which may reflect different C and N allocation strategies. Plants from the moraine developed a higher number of stolons than plants from the forest, which in turn had overall higher dry mass (Fig. 5). This suggests that *G. magellanica* plants behaving as early colonizers invest more energy in producing reproduction structures to quickly spread over the moraine. Moreover, the harsh conditions occurring close to the glacier (i.e. higher light intensity, wind exposure) may endure functional adaptations such as lower leaf size, higher SLA, higher net photosynthesis, etc. (Körner, 2003). Conversely, forest plants seem to invest in survival structures (e.g. thicker stolons, bigger leaves).

The lower photosynthetic performance of the studied lichens was not surprising, as lower photosynthetic rates than vascular species are profusely reported for cryptogams (Larcher, 1995). Previous laboratory analysis confirmed that our field measurements were performed under optimum conditions (De los Ríos *et al.*, 2011; Raggio *et al.*, 2012). This low photosynthetic capacity of cryptogams which are the dominant organisms at initial stages of primary colonization in Pia Glacier may explain the slight increment in soil C content during these initial stages (Arróniz-Crespo *et al.*, 2014). However, this situation contrasts again with

the extraordinarily high growth rate reported for the lichen *P. perrugosa* (thallus diameter growing rate of 20.4 mm·year^{-1}; Sancho et al. 2011).

Conclusions

Here we provide evidence of extraordinarily high N inputs during the primary succession in front of the receding Pia Glacier, as a consequence of both the high nitrogenase activity and dense coverture of the native herb *Gunnera magellanica*, endosymbiotically associated with cyanobacteria. Similarly, our results from $\delta^{15}N$ highlight that pioneer plant species potentially benefit from the efficient mechanism of acquisition and transfer of N throughout the succession provided by the symbiotic association with mycorrhizae. Thus, the system seems to successfully overcome the typical nutrient deficit of initial colonization stages and rapidly turns into a fertile habitat. Strikingly, the photosynthetic activity rates observed for the dominant plant species in the area did not resemble the high N input into the system, with rates similar to those reported in other temperate regions. This suggests that the fast growth rates observed are a consequence of efficient C allocation strategies instead of improved photosynthetic rates. Together, our work highlights the uniqueness of this system where symbiosis dominates the nutrient economy across the succession.

Supplementary material

Appendix S1

Calculation of different bottle headspaces for ARA measurement.

Calculations of headspaces were performed based on the volume occupied by each sample. *Gunnera magellanica* samples were carefully compressed after ARA measurements at the bottom of the incubation bottle and the height of the cylinder formed with its biomass was measured. We estimated *G. magellanica* sample volume by the cylinder volume formula ($V=\pi \cdot r^2 \cdot h$), being h the height of the biomass cylinder and r the circumference radius from the bottle section. Lichen sample volume was determined by measuring the displaced water volume by immersing their thalli (rock and thalli in case of *P. perrugosa*) in a graduated cylinder. Lichen thalli were previously hydrated until saturation to prevent water absorption by lichen thalli when determining their volume. Incubation headspace was then assessed by subtracting the sample volume from the volume of the incubation bottle.

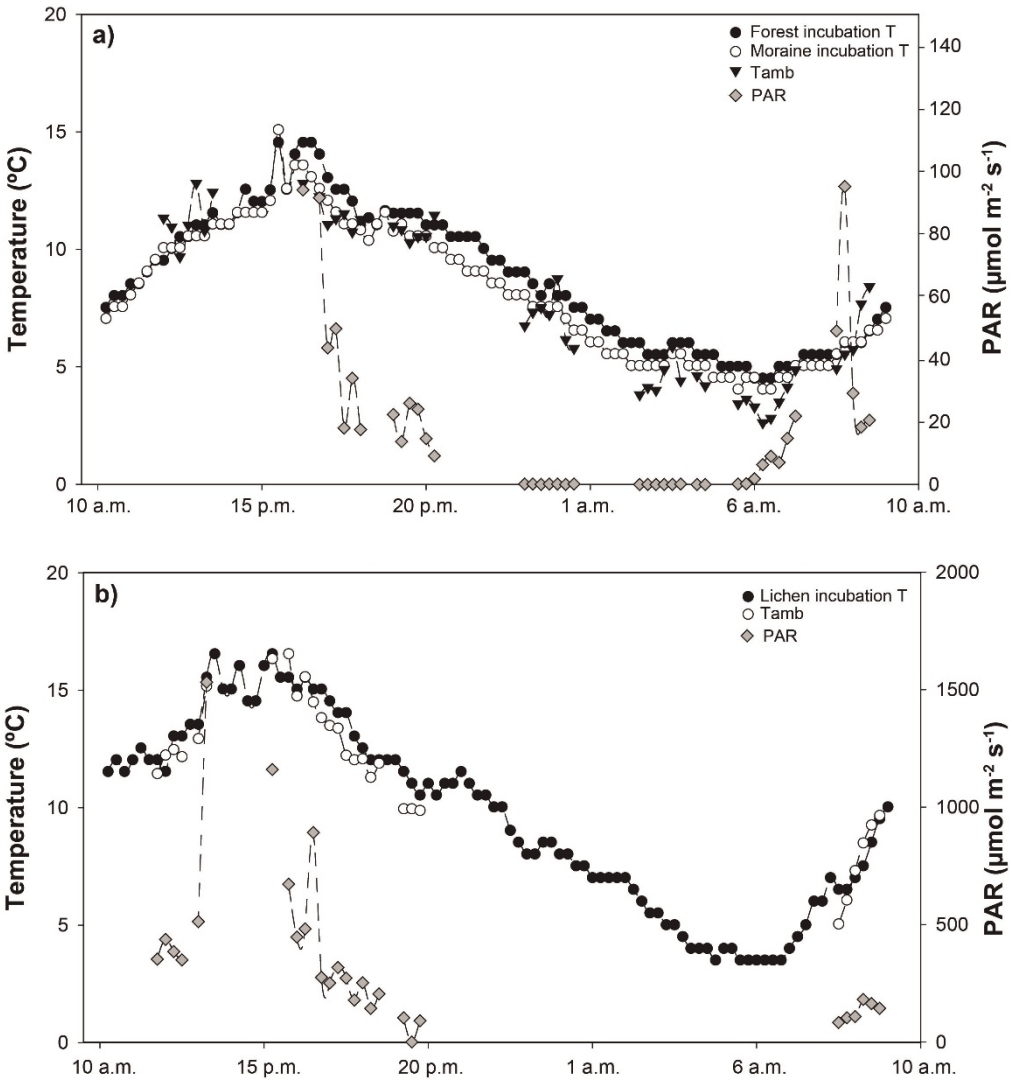
Table S1. Results of PERMANOVA pairwise post-hoc comparisons between nitrogenase activity results obtained for studied species and localities. GM: *Gunnera magellanica* (moraine); GF: *Gunnera magellanica* (forest); PE: *Peltigera patagonica*; PL: *Placopsis perrugosa*; ST: *Stereocaulon alpinum*. *P*-values below 0.05 are in bold. (n=5).

	Area		**Dry weight**	
	t	*P*	t	*P*
GMXGF	0.208	0.997	3.339	0.041
PEXPL	2.545	**0.004**	0.871	0.561
PEXST	2.885	**0.002**	3.034	**0.002**
PLXST	1.916	**0.031**	3.434	**< 0.001**

Table S2. Results of PERMANOVA pairwise post-hoc comparisons between net photosynthesis and respiration results obtained for studied species and localities. *P*-values below 0.05 are in bold (n=5).

Species	Locality	Photosynthesis		Respiration	
		t	*P*	t	*P*
G. magellanica	Moraine x Forest	2.448	**0.005**	0.804	0.502
	Forest x Control	0.540	0.965	1.073	0.332
	Moraine x Control	0.736	0.572	1.362	0.177
N. antarctica x G. magellanica		3.231	**0.005**	1.155	0.270
P. patagonica x P. perrugosa		0.708	0.480		

Figure S1. Environmental conditions inside and outside incubation bottles. a) *Gunnera magellanica* incubation; b) Lichens incubation. Tamb: outside bottle temperature; PAR: Photosynthetic Active Radiation.



CAPÍTULO 2: Elevational variation in morphology, nitrogen fixation and photosynthetic traits of *Gunnera magellanica* Lam. in a sub-Antarctic ecosystem

Abstract

Plant functional traits change as a consequence of contrasting environmental conditions, a phenomenon which is usually referred as phenotypic plasticity. These changes follow generally conserved patterns, with multiple morphological, physiological or developmental traits consistently affected across environmental gradients. Given the key implications of plant traits for ecosystem functioning, understanding their variation across environmental gradients is crucial to better understand the ecological impacts of climate change. Elevational gradients are commonly used to study the phenotypic plasticity of plant species, albeit they have barely been used in sub-Antarctic ecosystems so far. Here we evaluate the elevational (0-800 m) changes in multiple traits (related to plant morphology and physiology) and population characteristics of the Nitrogen (N) fixing herb *Gunnera magellanica* in three contrasting habitats (forest, *krummholz* and tundra) in the sub-Antarctic region of Tierra del Fuego, Chile. We found that *G. magellanica* plants showed a marked morphological trait adjustment (e.g. leaf lamina size or petiole length) to habitat conditions, but functional traits such as SLA, tissue N and photosynthetic pigment contents did not change with elevation. N fixation capacity diminished with elevation (on an apex basis) but was constant on dry weight basis. This species maintained an elevated N fixation regardless the habitat of 200 kg $N \cdot ha^{-1} \cdot yr^{-1}$, mostly as a consequence of higher plant density (number of plants per area) in tundra habitat. Altogether, our results show that, although *G. magellanica* shows considerable phenotype plasticity in some characters, it shows none in major functional traits like SLA, tissue N content or photosynthetic pigment content, which are usually highly plastic. The latter suggests that the N supply from fixation allows *G. magellanica* to maintain high C-fixation capacity at all sites and probably also make a major contribution to the nitrogen cycle.

Keywords: elevational gradient, nitrogenase activity, phenotypic plasticity

Introduction

Understanding how multiple morphological, physiological and developmental plant functional traits vary as a response to contrasting habitat conditions (i.e. phenotypic plasticity) has attracted substantial scientific attention in recent years (Sultan, 1995, 2000; Wilson *et al.*, 1999; Van Kleunen & Fischer, 2005; Valladares *et al.*, 2007; Gratani, 2014; Palacio-López *et al.*, 2015). This is especially true in relation to the study of the capacity of species to cope with ongoing global environmental change (Matesanz *et al.*, 2010; Nicotra *et al.*, 2010; Matesanz & Valladares, 2014; Wright *et al.*, 2016) or their invasiveness (Richards *et al.*, 2006; Hulme, 2008; Matesanz *et al.*, 2012; Monty *et al.*, 2013). In addition, the plasticity of functional traits such as specific leaf area (SLA), tissue nitrogen (N) concentration or drought tolerance (Funk et al. 2017) largely control the effects of plant species on processes that, such as litter decomposition or N fixation, largely determine ecosystem functioning (Faucon *et al.*, 2017).

Elevational gradients are often used as proxies to study the morphological or physiological adaptation mechanisms of species to the contrasting climatic conditions across their natural distributional range (e.g. Emery et al. 1994; Conover and Schultz 1995; Cordell et al. 1998; Fabbro and Körner 2004; Read et al. 2014). As elevational gradients are a surrogate for one or more environmental variables (e.g. temperature, radiation,; Rahbek 2005), they serve as natural experiments to characterize phenotypic plasticity within plants, and to anticipate the behavior of specific species, and consequent impacts on ecosystem functioning derived from community composition shifts under possible future climatic scenarios (Hillyer & Silman, 2010; Pepin *et al.*, 2015). Despite their interest and increased use within ecological studies, relatively few studies so far have used this approach in sub-Antarctic ecosystems (Barrera *et al.*, 2000), which are highly vulnerable to climate change (Thompson *et al.*, 2002; Pendlebury & Barnes-Keoghan, 2007).

The capacity of plants to fix atmospheric nitrogen (N) by establishing symbiotic relationships with diazotrophic bacteria is an important plant functional trait with paramount implications for ecosystem functioning (Batterman *et al.*, 2013; Blesh, 2018). N fixing species can incorporate a large amount of N into the soil, which promotes plant growth, and therefore carbon fixation and storage (LeBauer & Treseder, 2008; Batterman *et al.*, 2013). Moreover, when soil N is a limiting factor, N fixation also provides substantial advantages over their direct competitors (e.g. higher leaf nitrogen and phosphorus content, higher photosynthetic pigment and enzymes synthesis and consequently higher

photosynthetic capacity; Houlton *et al.*, 2008; Vergutz *et al.*, 2012; Adams *et al.*, 2016). N fixation is especially relevant in those regions where the low soil N availability constrains ecosystem productivity (Vitousek & Howarth, 1991), thus both nitrogenase activity and diversity of plants with this advantage would be expected to be higher in N limited ecosystems. There is a latitudinal distribution of N-fixation decreasing from the tropics to high latitude regions, with mature high-latitude forests paradoxically poor in N fixing species and N limited (Cleveland *et al.*, 1999; Menge *et al.*, 2014). Several environmental constraints (e.g. temperature, precipitation) can limit N fixation and provide theoretical support to this latitudinal pattern (Vitousek *et al.*, 2013). For the same reasons, elevational gradients are expected to affect nitrogenase activity (Sharma, 1988; Sharma *et al.*, 2010). Low temperature can negatively affect the establishment of the symbiotic relationship and the enzymatic activity, but increase the efficiency of the nitrogenase (Layzell *et al.*, 1984; Prévost *et al.*, 1987; Rai *et al.*, 2002; Lee & Son, 2005). However, adaptation to local conditions may also occur limiting the fitness of broad scale patterns of N fixation (Prévost *et al.*, 1987; Svenning *et al.*, 1991). Surprisingly, global N fixation estimations and assessments underpinning the abovementioned gradients mostly exclude non-actinorrhizal associations (e.g. endosymbiotic cyanobacteria; Cleveland et al. 1999) or do not consider the diazotrophic activity and regional variability of members of the Gunneraceae (Galloway *et al.*, 2004). This may be distorting our understanding on general patterns in N fixation in the southern hemisphere, where these species are widespread. Thus, understanding the N fixation patterns of these species is crucial to improve our understanding on global patterns in N fixation and N cycling.

Gunnera magellanica Lam. is a perennial deciduous herb, native to South America, recently proposed as a key species to Fuegian ecosystem functioning (Chapter 1). As a member of the family Gunneraceae, –the only angiosperm taxon known to establish endosymbiotic relationship with cyanobacteria (*Nostoc*; Osborne and Bergman 2009; Santi et al. 2013)–, *G. magellanica* is able to fix atmospheric N into plant assimilable forms (i.e. NH_4^+; Söderbäck *et al.*, 1990). In fact, this species possesses the highest N fixation activity within the family Gunneraceae measured so far (32.73 g N / m^2·yr; Chapter 1), which is also among the highest from nodulated phanerogam species (e.g. *Alnus*, *Casuarina*, *Acacia* and *Glycine*) and possibly only exceeded by a similar cyanobacterial symbiosis in the aquatic fern *Azolla* (Kellar & Goldman, 1979; Silvester, 1983; George *et al.*, 1988; Rennie *et al.*, 1988; Binkley *et al.*, 1994; Vaishampayan *et al.*, 2001). This high N fixation activity is expected to have a large

impact on the ecosystem (Chapter 1), especially in Tierra del Fuego where atmospheric N deposition is among the lowest on Earth (Dentener *et al.*, 2006). Moreover, *Gunnera* is a widespread genus in the southern hemisphere, including high-latitude regions such as Tierra del Fuego (Chile) and New Zealand (Wanntorp & Wanntorp, 2003). Thus, the contribution of this genus to alpine and high-latitude ecosystems may have important implications for N cycling and ecosystem functioning in these areas.

In Tierra del Fuego, *G. magellanica* thrives profusely in the lowlands, forming dense carpets under the forest canopy and in clearings. It is an early colonizer species able to survive under the harsh conditions of barren substrates and to speed up ecological succession (Sancho *et al.*, 2011; Arróniz-Crespo *et al.*, 2014; Pérez *et al.*, 2017). Additionally, *G. magellanica* has a wide elevational range from sea level to the timberline and the sub-Antarctic tundra, where it develops dwarf forms growing as mats of cushion plants (Moore, 1975). High phenotypic plasticity by *G. magellanica* may explain its broad ecological niche and to allow this species to withstand very harsh conditions (Sultan, 2000). Additionally, its wide ecological niche might help explain why some species of *Gunnera* are successful invaders (e.g. *G. tinctoria* and *G. manicata*; Osborne et al. 1991; Skeffington and Hall 2011; Gioria and Osborne 2013). There is also the possibility, as has occurred for other species with similar distributional preferences, of expansion into the maritime Antarctica (Smith & Richardson, 2010), a highly vulnerable region to invasions located just 900 km to the south; (Chown *et al.*, 2012). Thus, comprehensive approaches studying the phenotypic plasticity of *G. magellanica* across different habitat conditions may help to understand the strategies of this species to successfully survive over a broad range of environments.

To improve our understanding of the main mechanisms driving the successful adaptation of *G. magellanica* to contrasting habitat conditions, we assessed the variability of multiple functional and morphological traits of this species across an elevational gradient in the sub-Antarctic island of Navarino, Chile. We expect: (1) plants from the three different habitats will not only differ morphologically (e.g. leaf area) but also in functional traits (e.g. SLA, photosynthetic pigment or leaf nutrient contents) as a consequence of multiple adaptations to contrasting conditions; (2) *G. magellanica* plants growing under alpine tundra conditions will show lower N fixation activity than plants located at sea level as a consequence of lower average habitat temperatures.

Materials and Methods

Site description

The study site was located at the north central part of Isla Navarino, Chile (Fig. 1). The climate of Isla Navarino is dominated by a strong oceanity with cold summers (mean temperature from 8 to 11 ºC) and mild winters (mean temperature from -2 to 4 ºC). Mean annual temperature is c. 5.9 ºC at sea level (http://www.globalbioclimatics.org; Rivas-Martínez, & Rivas-Sáenz, 1996-2017) and c. 0.2 ºC at 800 m. However, maximum average temperature during the growing season is similar at both altitudes. The area is exposed to permanently westerly winds (Tuhkanen, 1992) and has one of the driest climates (ca. 449 mm/year) along the Beagle Channel (Santana *et al.*, 2006). The study area forms part of the Tierra del Fuego ranges composed of highly altered Paleozoic rocks, Jurassic metamorphic rocks and Cretaceous turbidites, all of which display plutonic intrusions (Olivero & Martinioni 2001, Menichetti et al. 2008). The vegetation is characterized by a marked elevational stratification (see Molina et al. 2016), with a relatively low tree line (550-650 m asl) as the most characteristic landscape feature of the region (Pisano 1977). Mature forests of evergreen and deciduous *Nothofagus* spp. dominate the island from sea level up to *krummholz* formation. Above timberline, the landscape is dominated by Magellanic (sub-Antarctic) tundra with pulvinate-cushion vegetation of *Bolax gummifera* and *Abrotanella emarginata*, among other taxa (Moore, 1975), and cryptogamic species such as *Usnea* spp. (Laguna-Defior, 2016; Molina *et al.*, 2016). *Gunnera magellanica* is distributed over the entire area.

Sampling design

Field sampling took place during the Austral summer season of 2016. *Gunnera magellanica* plants were collected from three habitats where this species was abundant: low elevation forests dominated by the evergreen *Nothofagus betuloides* and the deciduous *N. pumilio* and *N. antarctica* (hereafter forest habitat); mid-elevation dwarf and pure forests of *N. pumilio* (hereafter *krummholz* habitat); and tundra communities of *B. gummifera* and *A. emarginata* in small troughs with water runoffs (hereafter tundra habitat). Five populations with dense and extensive formations of *G. magellanica* and separated by at least 100 m between them were randomly selected within each habitat except for the *Krummholz*, were only four populations were selected due to the impossibility to find populations with enough distance between them. In order to accurately represent each population, ten samples per population

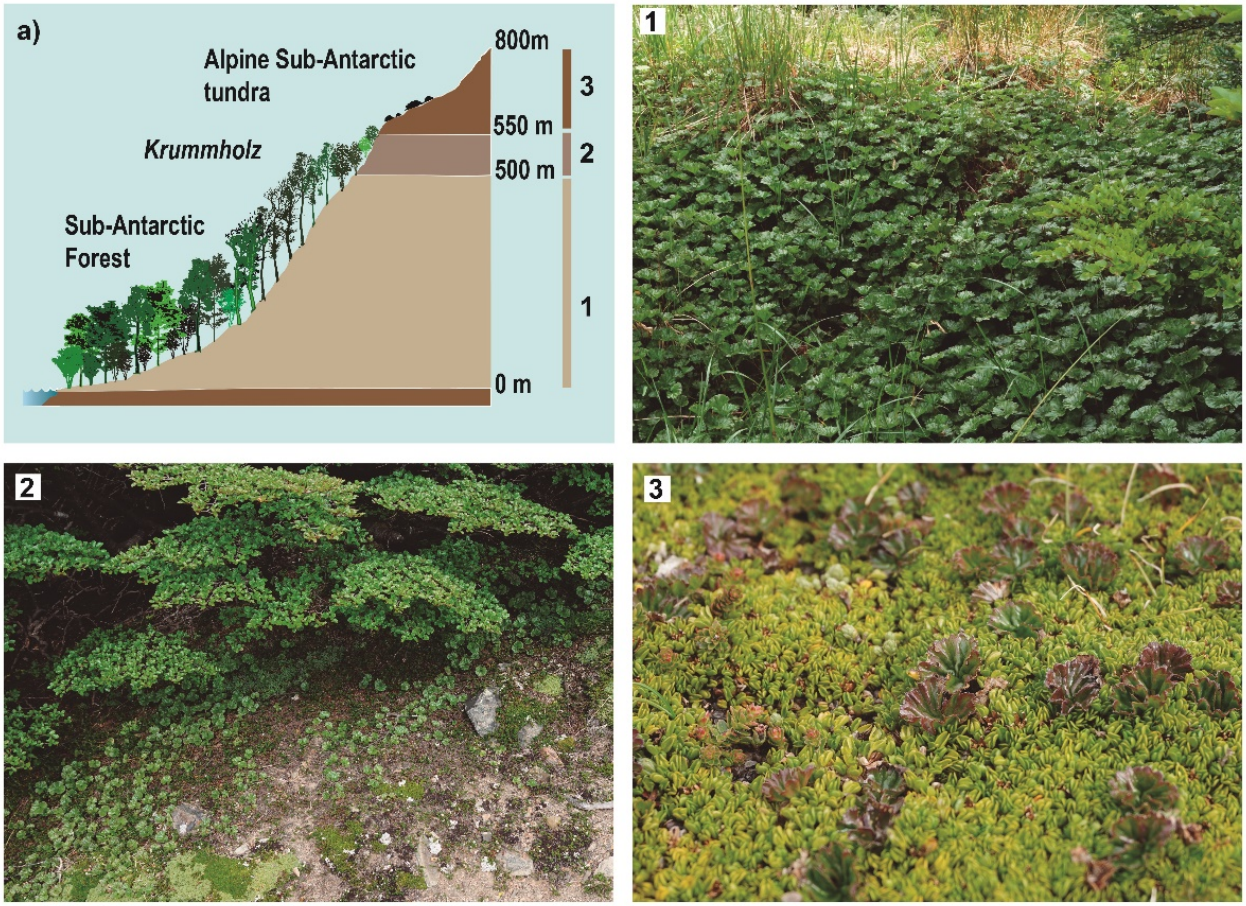


Figure 1: (a) Profile of the elevational transition of the major vegetation units in Navarino Island (Tierra del Fuego, Chile). Numbers represent the sampled habitats with abundant *Gunnera magellanica* populations: (1) forest habitat; (2) *krummholz* habitat; (3) tundra habitat.

were randomly collected keeping a distance of 2 m between them. All samples were collected on the same day and consisted of soil sections (15x15x10 cm) containing grown *G. magellanica* plants (including roots). These plants were transplanted to plastic pots, transported to the facilities of the Magallanes University in Puerto Williams, and placed in a shaded location (after watering) for subsequent N fixation and trait measurements within next 24h.

Trait measurements

Healthy adults of the plant species *G. magellanica* were measured during the most favorable season (ensuring maximal plant development) from the potted plants transported to the facilities of the Magallanes University in Puerto Williams. We measured multiple key functional traits related to plant morphology (rhizome, petiole and leaf), physiology (leaf SLA and CN content, photosynthetic pigments, and nitrogenase activity), and plant development (biomass) as well as population characteristic (plant density).

Morphological traits

Ten individuals of *Gunnera magellanica* were selected within each population, one per sample (potted plants). Each individual consisted of the terminal fragment of the rhizome (4 cm length; hereafter referred as apex) from which one adult leaf and its petiole was selected. The following traits were measured: apex thickness, fresh and dry petiole weight, petiole length, projected leaf area (LA), fresh and dry leaf weight. Petiole length was used as a surrogate for plant height due to the prostrate (rhizomatous) nature of *G. magellanica*. Fresh and dry weights were determined to 4 decimal places using a precision balance. Apex length and thickness and petiole length were determined with a caliper. Each leaf lamina was photographed lying on graph paper and projected area was obtained with ImageJ v. 1.51t software. Additionally, we calculated the specific leaf area (SLA) as the ratio of leaf area to dry mass.

Pigment and nutrient analyses

Collected leaves were divided in two halves, weighted, photographed and then immediately dried in a laboratory chamber (with forced air circulation) at room temperature (Kumar *et al.*, 2015), and transported to Spain for subsequent laboratory analysis. One half was used for pigment extraction using the dimethyl sulphoxide (DMSO) method of Hiscox and Israelstam (1979). Leaf fragments were incubated in DMSO for 30 minutes at 60 ºC and extracts were measured using a spectrophotometer (λ = 415, 435, 480, 648.2, 665, and 750 mm). The remaining halves of collected leaves were pulverized using a Precellys®24-DUAL lyser/homogenizer and encapsulated for total C and N determination with a CN analyzer (Leco CHN628 Series; Leco Corporation, St Joseph, MI, USA). The N concentration and content of leaf N was determined on both mass (N_{mass}) and area (N_{area}) bases, respectively.

Nitrogenase activity

Nitrogenase activity was estimated using the acetylene reduction assay (Hardy et al. 1968). Two composite samples per population were used for nitrogenase activity estimation, each sample consisting of three apexes (5 cm length) of *G. magellanica* rhizomes randomly selected from the plot set for each population. The rhizome apex of *G. magellanica* hosts the most active N fixation activity, which abruptly decays beyond the first 2 centimeters of the apex (Söderbäck *et al.*, 1990). Leaves, petioles and roots were removed before incubation and this has been shown not to alter the fixation capacity of *G. magellanica* in short-term experiments (i.e. 24 h assays; data not shown). Samples were introduced in wide-mouth glass

jars of 125 ml (for forest samples), and serum glass vials of 30 and 12 ml (for *Krummholz* and tundra samples, respectively), all fitted with a PTFE-faced silicone septum. Different volumes were required to ensure proportional headspace volume among sets, as apex thickness (and therefore apex volume) differed from one habitat to another. We used samples containing three different apexes to lower heterogeneity in nitrogenase activity between individuals without exponentially increasing the number of samples. Temperature outside and inside the glass bottles was continuously monitored during the incubation with iButton® dataloggers to verify that warming did not occur. One sample from each population was used as control (incubated without acetylene for basal ethylene production determination). The others were incubated with acetylene produced *in situ* by adding water to calcium carbide in a carbide lamp. Produced acetylene was stored in Tedlar® gas sampling bags until extracted for injection. 10% of the bottle volume was replaced by acetylene. After 1 hour incubation, a 5.7 mL sample was extracted using a gastight syringe and stored in 5.7 mL Exetainer® evacuated gas sampling vials. The process was repeated for each sample at three hour intervals for a 24-hour measuring cycle. Samples were aerated between incubations and samplings to prevent long-term incubation effects on nitrogenase activity (Dart & Day, 1971; David & Fay, 1977; Wani *et al.*, 1983), but continuously kept hydrated by spraying with deionized water. Gas samples were transported to Madrid for ethylene concentration determination using a GC: Varian 3300 gas chromatograph equipped with a J&W Agilent HP-PLOT Q column and a flame ionization detector. The N fixation rates were calculated from the ethylene concentration obtained from ARA by applying the most commonly used C_2H_4: N conversion factor of 3:1. Plant features (number of apexes·m^{-2} and dry weight) were used so that nitrogen fixation was finally expressed as nmols C_2H_4·g DW^{-1}·h^{-1}, µg N·g DW^{-1}·d^{-1} and kg N·ha^{-1}·yr^{-1}.

Statistical analyses

We first tested for significant differences in functional traits (excluding nitrogenase activity, biomass and plant density) from individuals of *G. magellanica* across habitats by conducting a semiparametric multivariate two-level ANOVA (PERMANOVA, Anderson 2001), with habitat as a fixed factor and population as random factor nested within habitat, and tested for differences among habitats for these variables using pairwise post hoc tests in PERMANOVA. Note that PERMANOVA allows unbalanced designs (i.e., different number of replicates) as in our sampling design. PERMANOVA analyses were developed using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymouth Marine

Laboratory, Ivybridge, UK). We followed a similar approach when testing for significant differences in biomass and plant density between habitats, but analyses were performed separately from functional traits because the different sampling procedure.

Measurements of N fixation were performed by repeatedly sampling the same individuals at specific intervals so that samples were not independent of each other; we therefore performed a repeated-measure analysis. To assess the significance of differences in N fixation rates of *G. magellanica* from different habitats surveyed we conducted a three-way PERMANOVA analysis (Anderson, 2001), based on Euclidean similarity matrix with habitat (three levels: forest, *krummholz* and tundra) treated as fixed factor, and time (7 levels) and population (five levels, nested within habitat) treated as random factors. Finally, we evaluated the correlation between the traits analyzed by using Spearman correlations conducted with IBM SPSS Statistics v22.

Table 1: Functional traits. Data are means ± SE (n = 5, except *Krummholz* with n = 4). Different letters represent statistical differences between habitats ($P < 0.05$ PERMANOVA) for a given trait. LA: projected leaf area; SLA: specific leaf area; SLM: specific leaf mass; DW:FW: dry weight to fresh weight ratio; LC: leaf total Carbon; LN: leaf total Nitrogen.

		Habitat		
Trait	**Units**	**Forest**	***Krummholz***	**Tundra**
Apex thickness	cm	1.002 (0.023)^{a}	0.673 (0.020)^{b}	0.569 (0.019)^{b}
Petiole length	cm	14.380 (0.667)^{a}	4.769 (0.198)^{b}	2.359 (0.137)^{c}
LA	cm^2	31.908 (1.487)^{a}	15.384 (0.755)^{b}	4.870 (0.316)^{c}
SLA	$cm^2 \cdot g^{-1}$	185.518 (3.274)^{a}	193.82 (4.742)^{a}	166.98 (6.601)^{a}
SLM	$g \cdot cm^{-2}$	0.055 (0.001)^{a}	0.053 (0.001)^{a}	0.065 (0.003)^{a}
Leaf DW:FW	-	25.120 (0.260)^{a}	22.115 (0.379)^{b}	24.022 (0.659)^{ab}
LC	%	44.107 (0.109)^{a}	43.664 (0.190)^{a}	42.882 (0.119)^{b}
LN	%	3.109 (0.056)^{a}	2.967 (0.045)^{a}	2.999 (0.074)^{a}
Leaf C:N	-	14.403 (0.254)^{a}	14.830 (0.201)^{a}	14.743 (0.386)^{a}
N_{area}	$g \cdot m^{-2}$	1.696 (0.041)^{a}	1.560 (0.039)^{a}	1.884 (0.062)^{a}
N_{mass}	$mg \cdot g$	31.086 (0.556)^{a}	29.667 (0.452)^{a}	29.997 (0.740)^{a}
Biomass (100% cover)	$Kg \cdot m^{-2}$	0.776 (0.082)^{a}	0.178 (0.029)^{b}	0.723 (0.084)^{a}
Plant density	Number apex $\cdot m^{-2}$	655.00 (73.80)^{a}	781.25 (92.29)^{a}	2984.08 (301.08)^{b}

Results

Morphological and community traits

Our results show that the traits of *G. magellanica* showed marked variation between the habitats ($P < 0.001$). Petiole length and LA showed a marked reduction from forest to tundra habitats (Table 1). Apex thickness similarly decreased with increased elevation, but this reduction was more evident (0.5 times thinner) between the pair Forest-*krummholz*, with slightly lower values for tundra individuals (0.2 times thinner) compared to *krummholz* plants. In contrast to LA, SLA and leaf C:N ratio were constant throughout all studied habitats (Table 1). Similarly, we found no differences between habitats for LN, N_{area} and N_{mass}, but significantly lower values for LC from tundra individuals compared to forest and

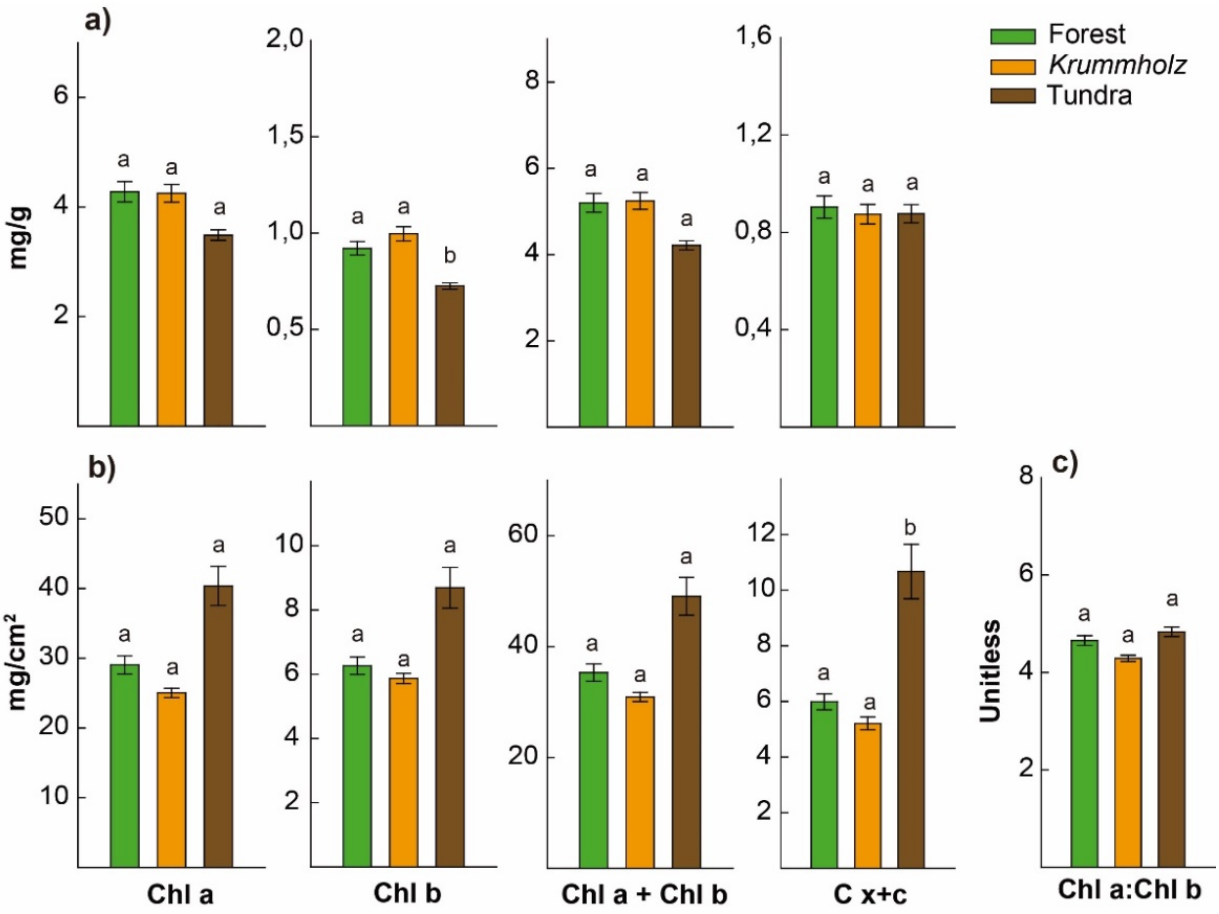


Figure 2: Photosynthetic pigment concentration (a) and content (b), as well as chlorophyll *a* and *b* ratio (c) of *Gunnera magellanica* plants from different habitats in Navarino Island, Chile. Bars represent means (n = 5, except *Krummholz* with n = 4) ± SE. Different letters indicate statistical differences ($P < 0.05$; PERMANOVA).

krummholz plants (Table 1).

For community traits, biomass was similar at forest and tundra sites, while *krummholz* plants had significantly lower biomass than the other habitats (P = 0.016, Table 1). Finally, the apex density (number of plants per area unit) was significantly higher in tundra site compared to forest and *krummholz* site (P = 0.008 and P = 0.016, respectively; Table 1).

Photosynthetic pigments

We also found differences between habitats in the photosynthetic pigments in *G. magellanica*. Although total photosynthetic pigment content (mg pigment per leaf dry weight) did not differ significantly between habitats, tundra plants had consistently lower contents than in forest and *krummholz* individuals, but only chlorophyll *b* contents were statistically significant ($P < 0.05$; Figure 2a). In contrast, pigment content on an area basis was always higher for *G. magellanica* plants from the tundra habitat, although these differences were only statistically significant for carotenoids ($P < 0.05$, Fig 2b). Chl*a*:Chl*b* ratios were similar for all samples from the studied habitats (Fig. 2c).

N fixation rates

We found marked diel variation in nitrogenase activity for *G. magellanica* in all the habitats studied, with peak and lowest values around midday (13-16 p.m.) and before sunrise (5 a.m.), respectively (Fig. 3). We did not find significant differences between habitats when N fixation rates were expressed per dry weight (P = 0.999). However, we found significant differences between habitats when nitrogenase activity was expressed per individual (i.e. apex). *Gunnera magellanica* rhizomes from the forest showed the highest nitrogenase activity, two and four times higher than in the krummholz and tundra, respectively (Table 2). We observed statistically significant differences between forest and tundra with *krummholz* plants when nitrogenase activity was expressed on an area basis. Forest and tundra plants showed similar average daily N fixation totals (41.07 and 56.06 nmol $C_2H_4 \cdot cm^{-2} \cdot h^{-1}$, respectively, Table 2), while *krummholz* plants showed significantly lower values (daily average of 28.07 nmol $C_2H_4 \cdot cm^{-2} \cdot h^{-1}$) than that of plants from the forest and the tundra (P = 0.047 and P = 0.008, respectively; Table 2).

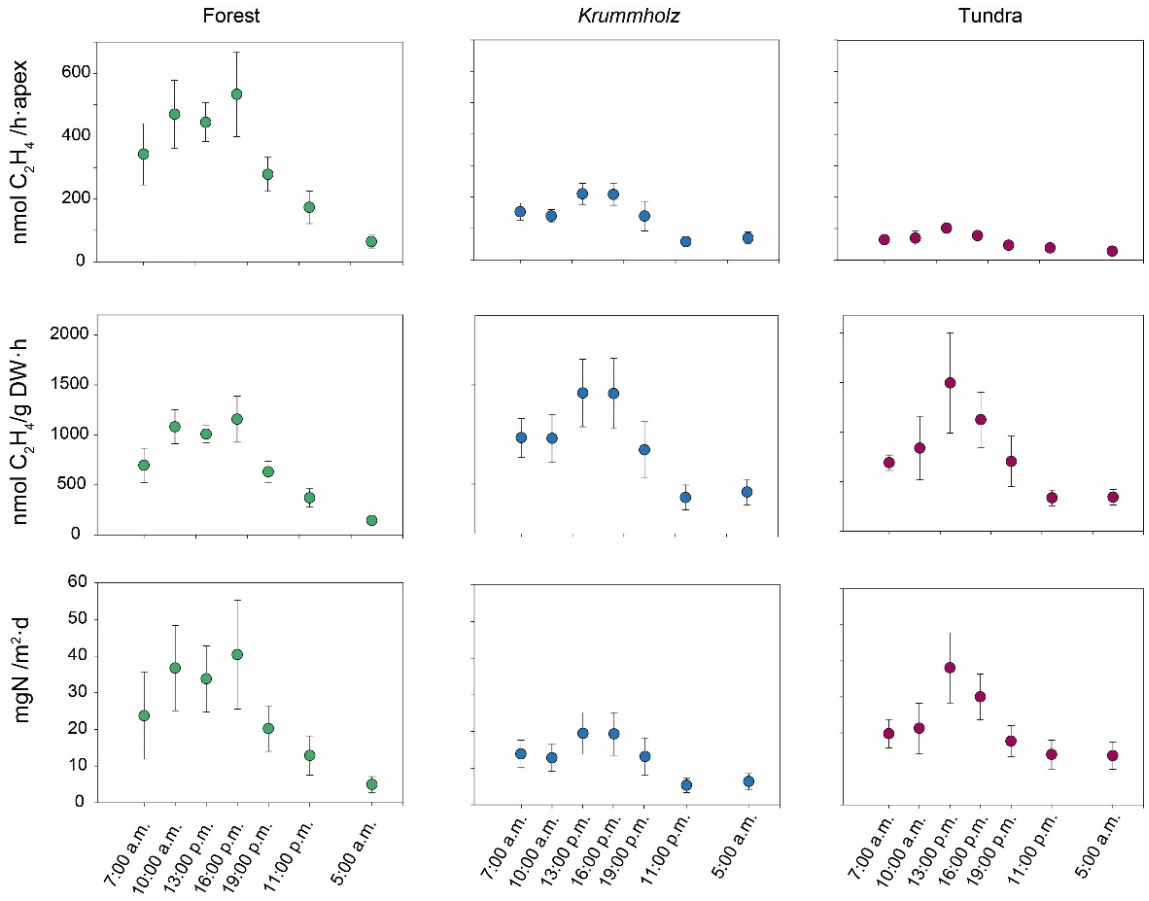


Figure 3: Nitrogen fixation rates under field natural conditions for *Gunnera magellanica* plants from different habitats in Navarino Island, Chile. Data are means (n = 5, except *Krummholz* with n = 4) ± standard error.

Table 2: N fixation rates and N contribution estimates for *Gunnera magellanica* plants from different habitats in Navarino Island, Chile. Rates were obtained from averaging daily measured cycles of nitrogenase activity measured by acetylene reduction assay. N contribution estimates were obtained using commonly reported C_2H_4:N conversion factor of 3:1. Data are means ± SE (n = 5, except *Krummholz* with n = 4). Different letters represent statistical differences between habitats ($P < 0.05$, PERMANOVA) for a given fixation rate.

Rates	Forest	*Krummholz*	Tundra
nmols C2H2 / g DW·h	1286.61 ± 139.42^a	1324.31 ± 171.1^a	1466 ± 206.37^a
nmol C2H2 / cm²·h	41.07 ± 4.91^a	28.07 ± 3.02^b	56.06 ± 5.07^a
nmol C2H4 /h·apex	275.14 ± 24.02^a	113.45 ± 9.56^b	61.93 ± 4.70^c
µg N / g DW·d	144.06 ± 15.61^a	146.45 ± 19.07^a	164.19 ± 23.11^a
g N / m²·yr	22.25 ± 2.5^a	11.48 ± 1.24^b	22.92 ± 2.07^a
kg N / ha·yr	222.5 ± 24.98^a	114.76 ± 13.36^b	229.16 ± 20.72^a

Discussion

Here we show that the functional traits of *G. magellanica* changed across the elevational habitat sequence evaluated in Navarino Island (Tierra del Fuego, Chile). More specifically, we observed large changes in morphological and community traits between plants from the forest, *krummholz* and tundra habitats, although some functional traits such as photosynthetic pigment content, SLA or N content were less responsive to habitat change across the elevational gradient studied. The N fixation capacity of *G. magellanica* was similar for all habitats in terms of plant dry mass, but the N contribution from different habitats was significantly lower in *krummholz*. This consistent N fixation capacity in such contrasting conditions might respond to different adaptations at community level. Our results highlight the morphological plasticity and the physiological robustness of *G. magellanica* in Navarino Island, which posits this species as a key species for ecosystem functioning in Tierra del Fuego.

Morphological and community traits

The variation in the traits LA, petiole length or apex thickness across the elevational gradient evaluated confirmed our initial hypothesis. These results are congruent with the profusely reported intraspecific morphological changes as a consequence of contrasting environmental conditions (Reich *et al.*, 1997; Milla *et al.*, 2009; Mclean *et al.*, 2014; Bongers *et al.*, 2017). Increasing elevation usually entails lower temperature, higher wind exposure, and higher precipitation, all factors affecting plant physiology and phenology (Körner, 2003; Gratani, 2014). LA and petiole length abruptly changed between evaluated habitats ($R^2 = 0.895$; $P < 0.001$; Table S1), with forest plants showing two and even six times bigger leaf lamina and three and six times longer petiole than observed in plants from *krummholz* and tundra habitats, respectively. Petiole length was used here as a surrogate for plant height, which is known to decrease with elevation (Fernández-Calvo & Obeso, 2004; Kichenin *et al.*, 2013; Mao *et al.*, 2018) and is ultimately related to light availability and competition strategies (Sakai, 1991; Falster & Westoby, 2003; Moles *et al.*, 2009), and also to physiological stress under harsh conditions (Körner, 2003). In this regard, the observed reduction in petiole length is consistent with reductions observed under high wind conditions, as such reductions minimize wind-induced mechanical damage (Niklas, 1996; Shen *et al.*, 2006). Long petioles in alpine Navarino habitats would translate into leaf damage in such a windy region (Tuhkanen, 1992). Similarly, LA reduction with increasing elevation has previously been observed in other species (Bresson *et al.*, 2011). Larger leaves usually respond to low light

conditions (Sultan, 2000), which are expected to occur under the forest canopy. Moreover, UV-B radiation had inhibitory effects on *G. magellanica*, reducing leaf growth when plants are exposed to ambient UV levels in Tierra del Fuego National Park (Argentina; Rousseaux et al. 2001). Thus, the absence of a protective canopy above *krummholz* may promote leaf reduction in Navarino Island associated to DNA damage (Rousseaux *et al.*, 1999; Giordano *et al.*, 2004).

Increasing elevation usually entails a reduction in SLA (or increase in SLM), with plants producing more robust leaves as acclimation to harsh conditions dominating alpine environment (Wright *et al.*, 2004; Poorter *et al.*, 2009; Scheepens *et al.*, 2010). However, we did not find statistical differences in SLA (or SLM) across habitats. Leaf traits such as SLA are usually among the most contrasting traits in terms of phenotypic plasticity (Wilson *et al.*, 1999; Shipley *et al.*, 2005; John *et al.*, 2017), but *G. magellanica* is known to maintain SLA (or SLM) while reducing leaf area under contrasting harmful radiation conditions (Giordano *et al.*, 2004), suggesting a low plasticity in SLA. These results do not support the general role of this trait in predicting growth and productivity within plants (i.e. the higher the SLA, the higher the relative growth rate and the productivity; Reich et al. 1997), which is expected to be larger in the forest than in the tundra. A similar lack of significant differences between habitats was surprisingly observed for leaf N content. The lack of variance observed for SLA (or SLM) and N_{mass} is consistent with the interdependence of these traits profusely reported in literature (Reich *et al.*, 1997; Wright *et al.*, 2004; Reich, 2014). This also suggests similar lifespan in plants from different habitats (Ryser, 1996; Poorter *et al.*, 2009), which would not match the expected longer length of the growing season in the lowlands than in the alpine habitat. A possible explanation for this lack of elevational differences in leaf N (and other related leaf traits) in *G. magellanica* may be the direct N supply from cyanobacterial endosymbionts, which may release the plant from its dependence on soil N availability (Craine *et al.*, 2009). In other words, direct N supply from its cyanobacterial counterpart may release *G. magellanica* of general constraints ruling leaf N economics.

Our results showed important differences in plant density (number of apices per area) between habitats. More specifically, plant density in the tundra was more than four times higher compared to populations under tree canopy. Although we did not analyze sexual reproduction, plants collected from forest populations frequently included fruiting spikes, and flowers in *krummholz* plants (A. Benavent, pers. obs.). However, no seeds or flowers were observed in tundra individuals. Investment in sexual reproduction is known to

decrease with elevation (Hautier *et al.*, 2009; Milla *et al.*, 2009) and vegetative reproduction is a common adaptation to harsh conditions in tundra habitats (Klimeš *et al.*, 1997; Carlsson *et al.*, 1999). This seems very clear for *G. magellanica* under tundra conditions in Navarino Island, where plants may persist investing energy in rhizome number production, with apical buds protected under the canopy of pulvinate-cushion species such as *Bolax gummifera* and *Abrotanella emarginata*) (Fig. 1; Moore 1975). However, plant biomass was similar in both forest and tundra habitats, which may suggest different C allocation strategies in contrasting habitats (C investment in leaf production and plant growth in the forest and in rhizome production and resistance in the tundra). A similar trade-off between vegetative growth and reproduction was suggested for this species while colonizing immediately deglaciated terrains, also dominated by harsh conditions (e.g. high radiation; Chapter 1).

Photosynthetic pigments

Photosynthetic pigments in *G. magellanica* leaves showed similar concentrations (mg pigment · g^{-1} DW) and content (mg pigment · cm^{-2}) between forest and *krummholz* habitats. However, tundra leaves showed slightly lower values for chlorophyll (*a* and *b*) concentration, but similar carotenoid concentration in all studied habitats. Conversely, we observed an opposite situation for pigment content, with tundra plants showing higher values in all habitats. This high carotenoid content may be a response to UV protection in alpine Tierra del Fuego, where ozone depletion allows elevated levels of UV-B during the growing season (Kirchhoff *et al.*, 1997; Rousseaux *et al.*, 1999). Carotenoids, besides accessory pigments for photosynthesis, are involved in photoprotection from potentially harmful photo-oxidative processes and chlorophylls damage (Bartley & Scolnik, 1995; Kovács & Keresztes, 2002). Moreover, carotenoid content is positively related to stress conditions of the plant (Peñuelas & Filella, 1998). Changes in photosynthetic pigment content above the timberline may couple the increment in flavonoids and other effects of higher UV radiation previously observed in *G. magellanica* (Ballaré *et al.*, 2001; Rousseaux *et al.*, 2001; Giordano *et al.*, 2003, 2004). In addition, the almost complete absence of significant differences in pigments between habitats is consistent with the conserved for SLA and leaf N, as both are tightly linked to photosynthetic capacity (Givnish, 2005; Funk *et al.*, 2017). Hence, our results suggest similar photosynthetic activity of *G. magellanica* plants at all studied habitats. Finally, it is noteworthy to mention the low correlation observed between leaf N_{mass} and photosynthetic pigment contents (Table S1). This result contrasts with the generally stated use of chlorophyll content as an indirect measure of nutrient status (Richardson *et al.*, 2002),

as leaf N is mostly invested in photosynthetic complex (Evans & Seemann, 1989). Here again, the diazotrophic activity of endosymbiotic cyanobacteria may affect this relationship in *G. magellanica*.

Nitrogenase activity

Our results show high N fixation capacity of *G. magellanica* across all evaluated habitats. However, habitat comparisons showed that nitrogenase activity of *G. magellanica* plants varied between sampled habitats in Navarino Island. These differences were only observed when expressed as net ethylene production per individual (i.e. nmol $C_2H_4 \cdot h^{-1} \cdot apex^{-1}$). Thus, our results confirm in part our second hypothesis (tundra individuals showing lower N fixation rates), as this lower nitrogenase activity seems not to occur because of a direct control of temperature on nitrogenase but rather indirectly by plant morphological and population adaptation to the harsh conditions of the tundra. Elevational changes in nitrogenase activity have previously been reported to be tightly coupled to variations in soil temperature and moisture (Sharma, 1988; Sharma *et al.*, 2010). However, when nitrogenase activity of *G. magellanica* was standardized using the dry weight of plant material, all habitats displayed similar rates. This is also surprising as *G. magellanica* plants from forest and tundra habitats in Navarino Island are known to host different *Nostoc* strains (Fernández-Martínez *et al.*, 2013). Our results suggest environmental filtering on plant selection of the symbiotic cyanobacteria strain, helping to maintain a required nitrogenase activity in the plant and adapt to local conditions as previously reported in other N fixing symbioses (Prévost *et al.*, 1987; Svenning *et al.*, 1991). Finally, our results suggest that apex thickness and consequently, *Nostoc* colony size have an important role driving nitrogenase activity, something previously observed for *Rhizobium* root nodules (Tajima *et al.*, 2007).

Our results confirm the high nitrogenase activity recently observed for this species in Pía Glacier foreland (Chapter 1). We acknowledge that our incubation methodology (just using rhizome apex and removing leaves and roots) prevents comparisons in N activity per dry weight, but the daily averaged N contribution observed in Navarino Island was in the same range (but slightly lower) than previously reported (Chapter 1). Nitrogenase activity is known to be highly dependent on water availability (Albrecht *et al.*, 1984; Sundstrom & Huss-Danell, 1987; Serraj & Sinclair, 1996). Thus, the lower fixation rate found in Navarino island compared to Pia Glacier foreland could be reflecting long-term plant water stress or suboptimal status under the much drier conditions of the eastern part of the Beagle Channel in comparison to the Pacific coast (432 mm. *vs.* ca. 2000 mm., respectively; Santana et al.

2006). Nevertheless, our results confirm *G. magellanica* as the most active N fixing species within the family Gunneraceae to date and among the most active non-nodulated N fixing species (Silvester & Smith, 1969; Becking, 1976). The large plant density (number of apices per area unit) observed in tundra counteracts lower individual N fixation, with forest and tundra habitats showing similar N contribution to the ecosystem. The large N inputs of *G. magellanica* at the tundra was unexpected, as alpine areas are expected to show low nitrogenase activity (Cleveland *et al.*, 1999).

Concluding remarks

Here we show that morphological traits (e.g. leaf lamina size or petiole length) of *G. magellanica* showed a marked adjustment to habitat conditions, but functional traits such as SLA, tissue N and photosynthetic pigment contents remained constant with elevation. Similarly, we found that this species maintained an unexpectedly elevated N contribution regardless the habitat of 200 kg $N \cdot ha^{-1} \cdot yr^{-1}$, which do not match general estimations claiming low nitrogen fixation in high-latitude temperate regions. However, we must note that worldwide estimations of N fixation are mostly based on trees, focusing only on mycorrhizal or actinorrhizal symbiosis (e.g. *Rhizobium* or *Frankia*), and endosymbiotic cyanobacteria are generally neglected (Cleveland *et al.*, 1999). In fact, to our knowledge any global or regional estimation of N fixation has included any *Gunnera* spp. among its dataset, which may be explained by the surprising lack of studies reporting the fixation rate of *Gunnera* spp. The Gunneraceae members are mostly present in the tropics and the southern hemisphere (Wanntorp & Wanntorp, 2003; Wanntorp *et al.*, 2004), and further estimates of their N fixation capacity and consequent N contribution could change the global estimations of N fixation, especially in high-latitude regions of the southern hemisphere (e.g. Patagonia).

Supplementary material

Table S1: Correlation coefficients (Spearman's rho) between all traits measured for *Gunnera magellanica* plants from different habitats in Navarino Island, Chile. Significant correlations are shown in bold. *: $P < 0.05$; **: $P < 0.01$. LA: projected leaf area; SLA: specific leaf area; SLM: specific leaf mass; DW:FW: dry weight to fresh weight ratio; LC: leaf total Carbon; LN: leaf total Nitrogen; A: area.

	Apex thick.	Petiole length	LA	DW:FW	SLA	SLM	LN	N_{area}	N_{mass}
Petiole length	**0.775****								
LA	**0.806****	**0.895****							
DW:FW	**0.202***	**0.221****	0.164						
SLA	0.103	**0.238****	**0.176***	**-0.675****					
SLM	-0.103	**-0.238****	**-0.176***	**0.675****	**-1.000****				
LN	0.087	0.060	0.060	**-0.404****	**0.452****	**-0.452****			
N_{area}	-0.011	**-0.208***	**-0.180***	**0.357****	**-0.554****	**0.554****	**0.321****		
N_{mass}	0.087	0.060	0.060	**-0.404****	**0.452****	**-0.452****	**1.000****	**0.321****	
LC	**0.351****	**0.403****	**0.419****	**0.304****	-0.082	0.082	0.110	**0.174***	0.110
Leaf C:N	-0.051	-0.024	-0.017	**0.445****	**-0.469****	**0.469****	**-0.984****	**-0.298****	**-0.984****
Chl a $_{DW}$	**0.187***	**0.317****	**0.328****	**-0.376****	**0.550****	**-0.550****	**0.445****	**-0.198***	**0.445****
Chl b $_{DW}$	**0.232****	**0.392****	**0.444****	**-0.249****	**0.392****	**-0.392****	0.160	**-0.262****	0.160
Chl a + Chl b $_{DW}$	**0.198***	**0.340****	**0.354****	**-0.354****	**0.532****	**-0.532****	**0.404****	**-0.210***	**0.404****
C x+c $_{DW}$	-0.069	0.066	-0.018	**-0.192***	**0.249****	**-0.249****	**0.215***	-0.042	**0.215***
Chl a $_{A}$	-0.120	-0.114	**-0.198***	0.081	-0.102	0.102	0.004	0.097	0.004
Chl b $_{A}$	-0.105	-0.075	-0.106	0.161	**-0.232****	**0.232****	**-0.298****	0.003	**-0.298****
Chl a + Chl b $_{A}$	-0.114	-0.111	**-0.186***	0.116	-0.138	0.138	-0.059	0.092	-0.059
C x+c $_{A}$	**-0.272****	**-0.243****	**-0.363****	0.135	**-0.228****	**0.228****	-0.084	**0.181***	-0.084
Chl a : Chl b	-0.048	-0.099	**-0.184***	**-0.210***	**0.281****	**-0.281****	**0.530****	0.144	**0.530****

Table S1. Continuation.

	LC	Leaf C:N	Chl a DW	Chl b DW	Chl a + Chl b $_{DW}$	C x+c $_{DW}$	Chl a $_{A}$	Chl b $_{A}$	Chl a + Chl b $_{A}$	C x+c A
Petiole length										
LA										
DW:FW										
SLA										
SLM										
LN										
N_{area}										
N_{mass}										
LC										
Leaf C:N	0.038									
Chl a $_{DW}$	0.081	-0.450**								
Chl b $_{DW}$	0.138	-0.160	0.862**							
Chl a + Chl b $_{DW}$	0.095	-0.407**	0.995**	0.901**						
C x+c $_{DW}$	-0.002	-0.223**	0.588**	0.454**	0.575**					
Chl a $_{A}$	-0.101	-0.033	0.360**	0.226**	0.343**	0.462**				
Chl b $_{A}$	-0.093	0.265**	0.234**	0.376**	0.261**	0.340**	0.825**			
Chl a + Chl b $_{A}$	-0.101	0.029	0.338**	0.255**	0.330**	0.454**	0.990**	0.883**		
C x+c $_{A}$	-0.136	0.061	0.082	-0.035	0.064	0.630**	0.803**	0.673**	0.803**	
Chl a : Chl b	-0.047	-0.530**	0.227**	-0.257**	0.148	0.294**	0.272**	-0.230**	0.182*	0.266**

CAPÍTULO 3: Plant community attributes predict elevational changes in microbial diversity, abundance and co-occurrence networks in a Sub-Antarctic environment

Abstract

Our understanding of the major environmental drivers controlling changes in soil microbial communities across elevational gradients lags behind that of plants and animals. While available evidence suggests that plant and animal diversity and abundance mostly follow a hump-shaped relationship, dropping off from mid to high elevations, it is unclear whether microorganisms consistently follow a similar trend. Here, we simultaneously evaluated how the diversity and abundance of multiple soil microbial groups (taxonomic and functional), and the major identified clusters in co-occurrence networks, changed across an elevational gradient (0-800 m) in the Sub-Antarctic island of Navarino (Chile). We also identified the environmental factors predicting observed elevation trends of soil microbial communities across the same elevational gradient. We found that increasing elevation strongly modified the diversity, abundance and co-occurrence network of multiple archaeal, bacterial and fungal taxa. However, we did not find a consistent trend for taxonomic (at multiple levels) and functional diversity along the elevational gradient studied. Conversely, the abundance of all microbial functional and taxonomic groups (except archaeal nitrifiers) consistently followed a hump-shaped relationship with elevation. Changes in habitat (from old-growth forests to alpine tundra), net primary productivity and plant species richness were the most consistent predictors of the variations observed in the diversity and abundance of soil microbes, but also in the relative abundance of taxonomic clusters within the soil co-occurrence network. These results emphasize the key role that plant community attributes play as drivers of elevational changes in the structure of soil microbial communities in Sub-Antarctic ecosystems. As such, plant attributes must be explicitly taken into account when studying microbial responses to changes in environmental conditions such as those found across elevational gradients.

Keywords: amoA; biodiversity; bacteria; fungi; microbial community; nitrogen cycle; nosZ; plant–soil (below-ground) interactions.

Introduction

The study of the distribution of plant and animal communities across elevational gradients (*sensu* McVicar & Körner, 2013) has fascinated biologists since the beginning of modern biogeography (von Humboldt & Bonpland, 1805; Darwin, 1859). Although elevational patterns in plant and animal diversity are not always consistent across the globe, available evidence suggests that plant and animal diversity mostly follows a hump-shaped relationship, with diversity of plant and animals dropping off from mid to high elevations (Vetaas & Grytnes, 2002; Grytnes, 2003; McCain, 2005; Rahbek, 2005). Much less is known, however, about how elevation regulates the diversity and abundance of soil microorganisms. Most recent studies –generally focused on bacteria– suggest that the diversity of soil microbes might also follow a hump-shaped or linear decline with elevation from regional to global scales (Bryant *et al.*, 2008; Fierer *et al.*, 2011; Delgado-Baquerizo *et al.*, 2016b). However, lack of relationships between microbial diversity and elevation, and increases in diversity with elevation have also been reported (Wang et al., 2017; Zhang, Liang, He, & Zhang, 2013). Such contrasting results are likely related to the different biotic and abiotic factors regulating microbial diversity across different environments. Identifying the key environmental predictors of microbial diversity within elevational gradients is, therefore, of paramount importance to advance fundamental ecological knowledge but also to develop appropriate management decisions.

Additionally, most studies evaluating elevational patterns in microbial diversity have focused on particular taxonomic groups, such as bacteria (Lipson, 2007; Singh *et al.*, 2012; Selmants *et al.*, 2016). Such limitation prevents us to properly understand the role of elevation in driving the diversity and abundance of multiple microbial taxa (Meng *et al.*, 2013; Siles & Margesin, 2016). Moreover, these studies have mostly focused on taxonomic diversity. Biodiversity is extremely complex, and involves components other than taxonomic richness (number of phylotypes) and diversity (Díaz *et al.*, 2011; Cernansky, 2017). Equally important, or even more important, for ecosystem functioning is the diversity of particular functional groups within microbial communities, such as nitrifiers (Cernansky, 2017). However, little is known on how changes in elevation regulate the functional diversity and abundance of soil organisms, particularly in Sub-Antarctic ecosystems, which are highly vulnerable to global environmental change.

Despite the growing interest in the network of interactions arising within microbial communities (e.g. De Menezes et al., 2015; Delgado-Baquerizo, Oliverio, et al., 2018a; Jones,

Hambright, & Caron, 2017), the major environmental drivers that control changes in the relative abundance of ecological clusters within these networks are poorly known. Soil microbes strongly co-occur, and form ecological clusters of particular taxa. These clusters represent important ecological units that provide the opportunity to identify the environmental preferences of highly connected and identifiable taxa (De Menezes *et al.*, 2015; Shi *et al.*, 2016; Delgado-Baquerizo *et al.*, 2018b). As expected for soil biodiversity, elevational gradients may result in significant changes in the co-occurrence network of soil microbes, albeit this has not been evaluated yet.

Multiple environmental factors might predict elevational changes in microbial diversity, abundance and co-occurrence networks. In fact, elevation is often used as a surrogate for one or more environmental variables that co-vary and directly impact species diversity (Rahbek, 2005). These variables include, but are not limited to, climatic factors (temperature and precipitation; Bahram, Põlme, Kõljalg, Zarre, & Tedersoo, 2012; Smith, Halvorson, & Bolton, 2002; Zhou et al., 2016) and soil properties (e.g., soil pH; Shen et al. 2013). For example, temperature, which often decreases with elevation, might affect microbial communities - sensitive to changes in temperature (Oliverio *et al.*, 2017)- via impacting metabolic aspects such as productivity and enzyme kinetics of major groups (Physiological tolerance hypothesis; Currie et al., 2004). Alternatively, other environmental drivers largely neglected in the current literature, such as vegetation community attributes (Prober *et al.*, 2015), might help to predict the distribution of microbial diversity and abundance across elevational gradients. For example, habitat change (e.g. from forest to tundra), plant productivity and diversity are well known to shift with elevation because of decreases in temperature. These changes might trigger modifications in microbial communities both directly, due to changes in the environmental preferences of their constituents, and indirectly, by altering resource availability (Lange *et al.*, 2015) and soil properties such as pH (a major driver of microbial communities; Fierer & Jackson, 2006). However, the role of plant attributes in regulating the relationship between soil microbial attributes and elevation remains largely unexplored. An integrative approach including multiple environmental drivers as predictors of taxonomic and functional diversity, abundance and co-occurrence networks across elevational gradients could help us to predict potential impacts of climate change on microbial communities, which are key for the provision of multiple ecosystem services (Bardgett & van der Putten, 2014).

Here, we provide the first integrated study evaluating elevational patterns (0 to 800 m) in taxonomic and functional diversity, abundance and co-occurrence network of soil microbes in the Sub-Antarctic region of Tierra del Fuego (Chile). This region is the closest forested area to the Antarctica, which is located just 900 km to the south. This short distance separates two contrasting continents with different climatic characteristics. In addition, vegetation dramatically changes with elevation in Tierra del Fuego, from old-growth forests to alpine tundra dominated by cryptogamic species, where only scattered and small vascular plants, some of them also present in Antarctica, can be found (see Molina *et al.*, (2016) for further description). These abrupt elevational shifts within a relative small area (7 km from sea level to the summit) make this region especially relevant for studying the effect of elevation on soil biodiversity. Previous studies in Tierra del Fuego have shown that the diversity of plants and stream macroinvertebrates follows a hump-shaped trend peaking at mid elevations, just before the ecotone between forest and tundra (Contador *et al.*, 2015; Molina *et al.*, 2016), but soil microbial diversity remains to be explored in this area. To fill this gap in our knowledge, we examined how microbial abundance, diversity and co-occurrence networks changed across elevational gradients, and identified the key environmental predictors explaining such a pattern. We hypothesized that: (1) as previously observed for plants, elevational patterns will led to drastic changes in the diversity, abundance and clusters within the co-occurrence network of microbial communities, decreasing with elevation; and (2) given the important changes in vegetation observed across this elevational gradient, habitat change will be a key driver of soil microbial communities due to the tight relationships between vegetation attributes and microbes (e.g., symbiotic relationships or belowground C allocation); and (3) microbial functional groups will show divergent elevational trends due to their resource requirements (e.g. organic matter availability) or contrasting climatic preferences (e.g. bacterial vs. archaeal nitrifiers). Finally we explored the link between microbial diversity and abundance with multiple single soil functions, to further evaluate the link between shifting microbial attributes and soil functioning.

Materials and Methods

Study site and sampling design

This study was conducted at the Cerro Bandera hill in the north-central part of Isla Navarino (54°55'- 55°20'S; l67°05' – 68°22'W, Fig. 1), located at the southern part of the Beagle Channel (Región de Magallanes, Chile). Seventeen sampling stations (1 x 30 m bands) where

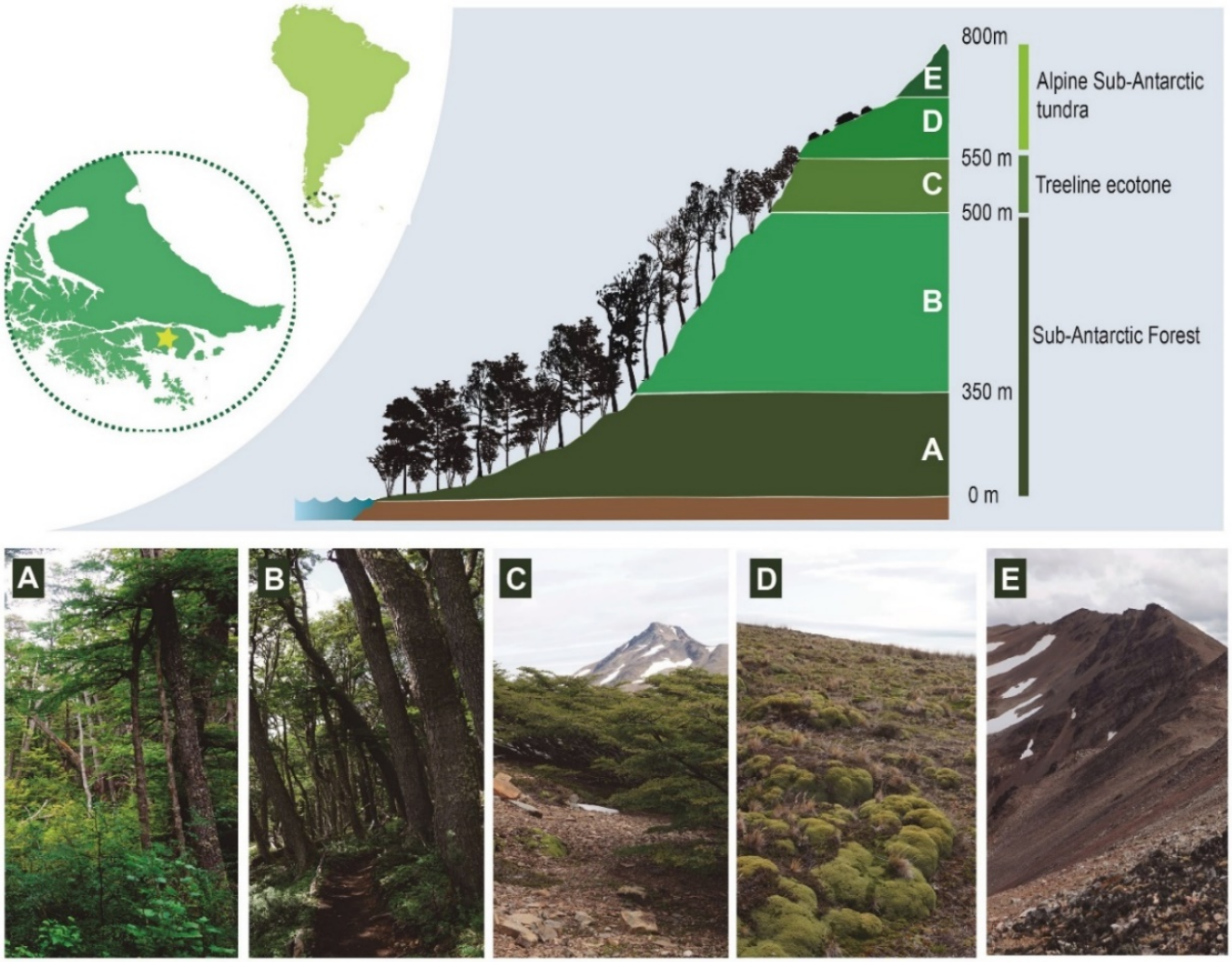


Figure 1: Map of sampling locations in Navarino Island, showing the major vegetation changes across the elevational gradient studied. A: mixed evergreen and deciduous forest of *Nothofagus pumilio* and *N. betuloides*; B: deciduous forest of *N. pumilio*; C: *Krummholz* of *N. pumilio*; D: cushion-like vegetation in Sub-Antarctic tundra; E: Alpine Sub-Antarctic tundra dominated by lichens.

uniformly distributed every 50 m along a 7 km elevation transect ranging from 0 to 800 m above sea level. Vegetation ranged from old growth sub-Antarctic forest (likewise subdivided in evergreen and deciduous forests dominated by *Nothofagus betuloides and N. pumilio,* respectively) at sea level up to sub-Antarctic tundra (Fig. 1). The climate of Isla Navarino is dominated by a strong oceanity with cold summers (mean temperature from 8 to 11 ºC) and mild winters (mean temperature from -2 to 4 ºC). Mean annual temperature is ca. 5.9 ºC at sea level (http://www.globalbioclimatics.org; Rivas-Martínez, & Rivas-Sáenz, 1996-2017) and ca. 0.2 ºC at 800 m. The area is exposed to permanently westerly winds (Tuhkanen, 1992) and has one of the driest climates (ca. 449 mm/year) in the area of Beagle Channel (Santana *et al.*, 2006). Further description of vegetation and bioclimatic features are reported by Molina et al. (2016). From a geological viewpoint, most of the area forms part of the Tierra del Fuego ranges composed of highly altered Paleozoic rocks, Jurassic metamorphic rocks and Cretaceous turbidites, all of which display plutonic intrusions (Olivero & Martinioni, 2001; Menichetti *et al.*, 2008).

Soil samples were collected during the austral summer season of 2015. At each sampling station, a composite sample was obtained by mixing the soils from 20 soil cores (5 cm depth), with a total of 17 soil samples along the transect. When sampling, particular effort was taken to avoid stones, fallen trees and wood debris. Collected soil samples were sieved with a 2 mm sifter and divided in two fractions. A fraction was immediately frozen at -20 ºC for assessing the abundance and diversity of broad and functional groups of microorganisms. The other fraction was air dried for biogeochemical analyses. Both fractions were transported to Rey Juan Carlos University in Móstoles (Spain) for laboratory analyses.

Assessment of microbial diversity and abundance

We extracted DNA from 0.5 g of defrosted soil fractions using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) following manufacturer instructions. We checked the quantity and quality of extracted DNA using a NanoDrop® ND-2000c UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The abundances of the bacterial (16S rRNA) and fungal (ITS) genes and of the functional genes *amoA* –for ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)– and *nosZ* (encoding the catalytic subunit of the nitrous oxide reductase from denitrifying prokaryotes; DNP) were analyzed using real time PCR (qPCR) on a CFX-96 thermocycler (Biorad, USA). Total bacterial 16S and fungal ITS genes were quantified using primer pairs Eub 338-Eub 518 and ITS1F-5.8s, respectively, following Evans and Wallenstein (2012). Primer pairs

CrenamoA23f/CrenamoA616r (Tourna *et al.*, 2008), amoA-1F/amoA-2R (Rotthauwe & Witzel, 1997) and nosZ2F/nosZr (Henry *et al.*, 2006) were used for quantifying *amoA* for AOA and AOB, and *nosZ* genes, respectively.

The taxonomic diversity of bacteria and fungi was characterized by amplicon sequencing (Illumina Miseq) using the 341F/805R (bacterial 16S rRNA gene, (Herlemann *et al.*, 2011) and FITS7/ITS4 (fungal ITS2 region, Ihrmark et al., 2012) primer sets in the Next Generation Sequencing Facility of Western Sydney University. The functional diversity of *amoA* and *nosZ* genes was characterized by terminal-restriction length polymorphism (T-RFLP) analysis. Amplicons for T-RFLP were produced using the fluorescently-labelled primer sets FAM-CrenamoA23f/CrenamoA616r, VIC-AmoA1F/AmoA2R and VIC-nosZ1211F/nosZ1719R, respectively, following Hu et al. (2015). PCR products were purified using the FavorPrep™ GEL/PCR purification Kit (Favorgen) according to manufacturer´s instructions. The concentration of purified DNA was measured with a NanoDrop spectrophotometer (see above), and the estimated concentration of DNA was used to normalize the DNA amount used for digestion. Restriction digests were performed using the restriction enzymes RsaI for AOA and AOB and MspI for nosZ (BioLabs, Sydney, NSW, Australia). T-RFs were resolved on an ABI PRISM 3500 Genetic analyzer (Applied Biosystems, CA, USA). A GeneScan 600-LIZ internal size standard (Applied Biosystems) was applied to each sample. T-RFLP profiles were analyzed using the GeneMapper software, version 4.0 (Applied Biosystems). Raw data from GeneMapper were analysed with T-REX, an online software for the processing of T-RFLP data (http://trex.biohpc.org; (Culman *et al.*, 2009). We used the approach outlined in Nazaries et al. (2013) for processing T-RFLP data. Briefly, quality control procedures included noise filtering and T-RF alignment (clustering threshold 2 bp), and T-RFs were omitted if they occurred either in less than 2% of the total number of samples or with a relative abundance of less than 1% within a specific sample.

We assessed the quality of all Illumina R1 and R2 reads using FastQC (Andrews, 2010). Low quality regions (Q < 20) were trimmed from the 5' end of the sequences (26 bp from R1 and 91 bp from R2 for primer set 341F/805R; 10 bp from R1 and 55 bp from R2 for primer set FITS7-ITS4R) using SEQTK (https://github.com/lh3/seqtk). The paired ends were subsequently joined using FLASH (Magoč & Salzberg, 2011). Primers were removed from the resulting sequences using SEQTK and a further round of quality control was conducted in MOTHUR (Schloss *et al.*, 2009) to discard short sequences (<380 bp for primer set 341F-805R; <171 bp for primer set FITS7-ITS4R), as well as sequences with ambiguous characters

or more than 8 homopolymers. Operational Taxonomic Units (OTUs) were built at 97% sequence similarity using UPARSE (Edgar, 2013). Singletons were discarded, as well as chimeric sequences identified by the UCHIME algorithm using the recommended SILVA gold 16S rRNA gene or UNITE reference databases for bacteria and fungi, respectively (Edgar *et al.*, 2011). OTU abundance tables were constructed by running the 'usearch_global' command and 'uc2otutab.py' script (http://www.drive5.com/). Taxonomy was assigned to OTUs in MOTHUR using the naïve Bayesian classifier (Wang, Garrity, Tiedje, & Cole, 2007) with a minimum bootstrap support of 60% and the Greengenes database version 13_8 (DeSantis *et al.*, 2006; McDonald *et al.*, 2012) for bacteria, or the dynamic UNITE version 6 dataset (Kõljalg *et al.*, 2013) for fungi. The OTU abundance tables were rarefied to an even number of sequences per sample (24880 and 31507 sequences for bacteria and fungi, respectively) prior to calculating α-diversity metrics using MOTHUR (Schloss *et al.*, 2009). The species richness (α-diversity) was calculated as the number of phylotypes (OTUs for amplicon sequencing and T-RFs for T-RFLP methods) at each site for each microbial taxon from the rarefied OTUs or T-RFs tables.

Climatic, productivity and soil characteristics

Air temperature and humidity at each station were measured at 3-hour intervals during one year using DS1923-F5 Temperature/humidity iButton® dataloggers located at 15 cm above soil surface in areas without direct sunlight exposure. Data from these sensors were processed to obtain the average daily temperature (T), average of minimum daily temperatures (Tmin) and average of maximum daily temperatures (Tmax). Data for net primary productivity (NPP; g C m^{-2} d^{-1}) were calculated using the Normalized Difference Vegetation Index (NDVI), which has been shown as a good estimator of above-ground net primary productivity (Tucker *et al.*, 1983; Prince, 1991; Paruelo *et al.*, 1999). NDVI data for each sampling site were acquired using the platform Google Earth Engine (https://earthengine.google.com). For the pixel centered on each sampling site location we extracted the median value of NDVI from the Landsat 8 OLI sensor Surface Reflectance product (pixel size of 30 m × 30 m) for the period 1 April 2014 to 31 March 2015. These images are atmospherically corrected using LaSRC (Landsat 8 Surface Reflectance Code) method, and include a cloud, shadow, water and snow mask produced using the CFMask algorithm. Plant richness data was obtained from Molina et al. (2016).

We measured soil pH for all of the soil samples with a pH-meter in a 1:2.5 mass/volume soil and water suspension. Sand, clay, and silt content were analyzed according

to Kettler et al. (2001). Electrical conductivity was determined using a conductivity meter in the laboratory. Soil water holding capacity (WHC) was determined by gravimetry. Soil total C and N were measured with a CN analyzer (Leco CHN628 Series; Leco Corporation, St Joseph, MI, USA) Organic C was determined following Anderson & Ingram (1993).

Soil functions

For each soil sample, we measured a wide range of surrogates of ecosystem functions linked to the stocks and cycling of C [dissolved organic C (DOC), α-Glucosidase (AG, starch degradation), β-Glucosidase (BG, starch degradation), β-D-cellobiosidase (CB, cellulose degradation), Xylosidase (XYL, hemicellulose degradation)], N [total N (TN), available N (AN), dissolved organic N (DON), NH_4^+-N, NO_3^--N, potential net mineralization and nitrification rates, microbial biomass N (MB-N), L-Leucine- aminopeptidase (LAP, protein degradation), N-acetyl-β-glucosaminidase (NAG, chitin degradation)], and P [available inorganic P (PO_4^{-3}, AIP), Phosphatase (PHOS, P mineralization)]. See supplementary Appendix 1 for methods description.

Co-occurrence networks

We generated two correlation networks, i.e. co-occurrence network, including (1) taxonomic and (2) functional microbial attributes. Both networks were created following the approached explained in (Delgado-Baquerizo *et al.*, 2018b). In brief, we kept those taxa accounting for more than 80% of the relative abundance of bacteria and fungi (taxonomic network) and AOA, AOB and DNP (functional network). These analyses were done independently for each taxonomic and functional group. We then merged relative abundance information for each group within two datasets: taxonomic and functional taxa. We then calculated all pairwise Spearman's rank correlations (ρ) between all soil taxa (OTUs and T-RFs for taxonomic and functional network, respectively). We considered a co-occurrence to be robust if the Spearman's correlation coefficient was > 0.50 and $P < 0.01$ (see Barberán, Casamayor, & Fierer, 2014 for a similar approach). A biological and mathematical explanation for this threshold is available in (Delgado-Baquerizo *et al.*, 2018b). The network was visualized with the interactive platform gephi (Bastian *et al.*, 2009). Finally, we used default parameters from this platform to identify ecological clusters of soil taxa strongly interacting with each other. We then computed the relative abundance of each cluster by averaging the standardized relative abundances (z-score) of the taxa that belong to each cluster. By standardizing our

data, we ruled out any effect of merging data from different soil microbial taxa (e.g. fungi and bacteria).

Fungal functional life styles

Information on functional life styles (e.g., saprobe, mycorrhiza, etc.) for fungal taxa across the entire ITS dataset was obtained from the online application FUNGuild described in Nguyen et al. (2016). The relative abundance of each functional group was calculated as the sum of the relative abundance of all phylotypes sharing that particular functional group.

Statistical analyses

We first evaluated the relationship between elevation and taxonomic/functional microbial richness, abundance, and ecological clusters by using polynomial regression analysis with the vegan package (v 2.4-6; Oksanen, Blanchet, Kindt, Legendre, & O'Hara, 2016) in R v3.3.2 (R Development Core Team, 2008). In particular, we identified the shape of the relationship between elevation and diversity using linear and quadratic functions, as they are the most common shapes reported for elevational biodiversity patterns, including positive/negative and concave/convex relationships. When parametric assumptions could not be ensured, model significance was calculated using permutation analysis (LmPerm package v2.1.0; Wheeler & Torchiano, 2016). We selected the best model fit in each case by following the Akaike Information Criterion (AIC_c; Burnham & Anderson, 2010). The lower the AIC_c value the better the model. Here, we consider $\Delta AIC_c > 2$ as the threshold to differentiate between two different models and then select that with the lowest AIC_c (Burnham & Anderson, 2002). When two models were similar (i.e. $\Delta AIC < 2$) we then selected the linear one.

We used Random Forest analysis (Breiman, 2001) as explained in Delgado-Baquerizo et al. (2015) to identify the best environmental predictors of taxonomic and functional microbial diversity, abundance ecological clusters and fungal functional groups. Previous to these analyses, and given the large number of environmental predictors in our dataset, we first removed those variables that suffered from multicollinearity. In particular, we removed climatic and environmental variables that were highly correlated to each other ($R^2 > 0.8$; Katz, 2011; Table S1). Variables showing a low collinearity or smaller variance were selected for further analyses. The following variables were not considered in Random Forest analysis: Elevation (high correlation with EC, T, Tmin and NPP), EC (high correlation with Tmin and NPP), soil N content (high correlation with soil WHC and soil C content), and mean temperature (high correlation with Tmin and NPP). Tmax was initially considered as a

predictor but finally discarded due to its consistent lack of significance and negative effect on model quality for all microbial attributes. Therefore, our Random Forest analyses included the next variables: soil total C, soil C:N ratio, soil WHC, NPP, pH, plant richness and Tmin. Our Random Forest model also included habitat change a 0/1 variable including information on the presence of forest (1) and tundra (0) in our environmental gradient. Following Random Forest analyses, we explored the shape of the relationship between microbial richness and abundance, ecological clusters and functional groups and their significant predictors by means of polynomial regression analysis, as specified above for elevation.

We evaluated elevational patterns in microbial community composition at multiple taxonomic levels. We first summarized the variation in the community composition (β-diversity) of the taxonomic and functional groups analyzed by means of non-metric multidimensional scaling (nMDS). We conducted nMDS ordinations with PRIMER v6 statistical package for Windows (PRIMER-E Ltd., Plymouth Marine Laboratory, UK), using the Bray-Curtis distance for the resemblance matrix (MiSeq and T-RFLP results for taxonomic and functional groups, respectively) and 25 re-starts to calculate stress values (stress < 0.1 in all cases). We then evaluated the relationship between the first axis of the nMDS and elevation by means of polynomial regression analysis. Second, we evaluated the links between elevation and taxonomic and functional diversity and relative abundance of major phyla (those accounting for more than 95 % of total abundance) by means of polynomial regression analysis. Model fit selection followed previous description (AIC_c).

Finally, we evaluated the link between taxonomic and functional diversity and abundance with the soil functions measured by means of correlation analysis. As parametric requirements were not fulfilled for all measured soil variables, we performed Spearman rank correlation analysis.

Results

Ecological drivers of taxonomic and functional microbial diversity and abundance.

A total of 7,795 (fungi) and 17,579 (bacteria) operational taxonomic units (OTUs) from amplicon sequencing and 46 (AOA), 92 (AOB) and 35 (DNP) terminal restriction fragments (T-RFs) were characterized along the elevational gradient studied. The diversity of fungi and bacteria followed opposite elevational patterns. In particular, we found that bacterial diversity followed a marked hump-shaped relationship (Fig. 2a), while fungal diversity mostly increased with elevation in a non-linear way (Fig. 2b). The richness of AOB linearly

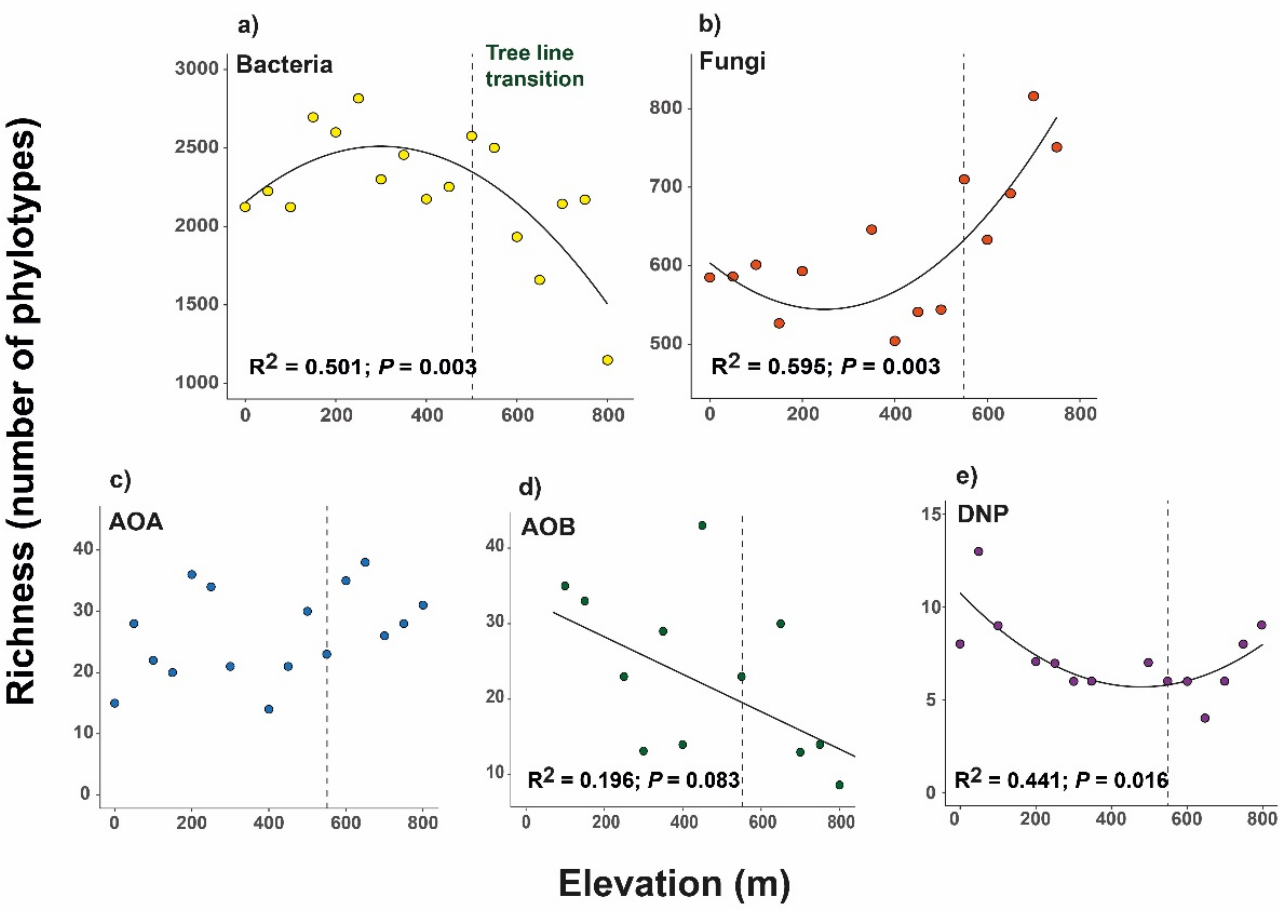


Figure 2: Taxonomic and functional richness (number of OTUs and T-RFs, respectively, obtained from Illumina MiSeq sequencing of fungal ITS and bacterial 16S amplicons and T-RFLP functional *amoA* and *nosZ* groups) along the elevational gradient studied. Model fit statistics and AICc values describing the relationship between elevation and the richness of taxonomic and functional groups are available in Table S2. AOA: ammonia oxidizing archaea; AOB: ammonia oxidizing bacteria; DNP: denitrifying prokaryotes.

decreased with elevation (Fig 2d), while we did not detect any significant relationship between the richness of AOA and elevation (Fig 2c). Finally, the richness of DNP species showed a convex response (Fig. 2e). Hump-shaped responses were mostly observed for all the above mentioned groups when evaluating the relationships between their abundance and elevation, excepting for AOA, which was not related to elevation (Fig 3). The importance of the environmental predictors evaluated varied depending on the microbial attribute considered, albeit habitat change, plant species richness and net primary productivity were particularly important and often selected by our Random Forest models (Fig. 4). A summary of the best models for the regressions between microbial groups and their predictors are shown in Table 1 (statistics provided in Tables S2-S4).

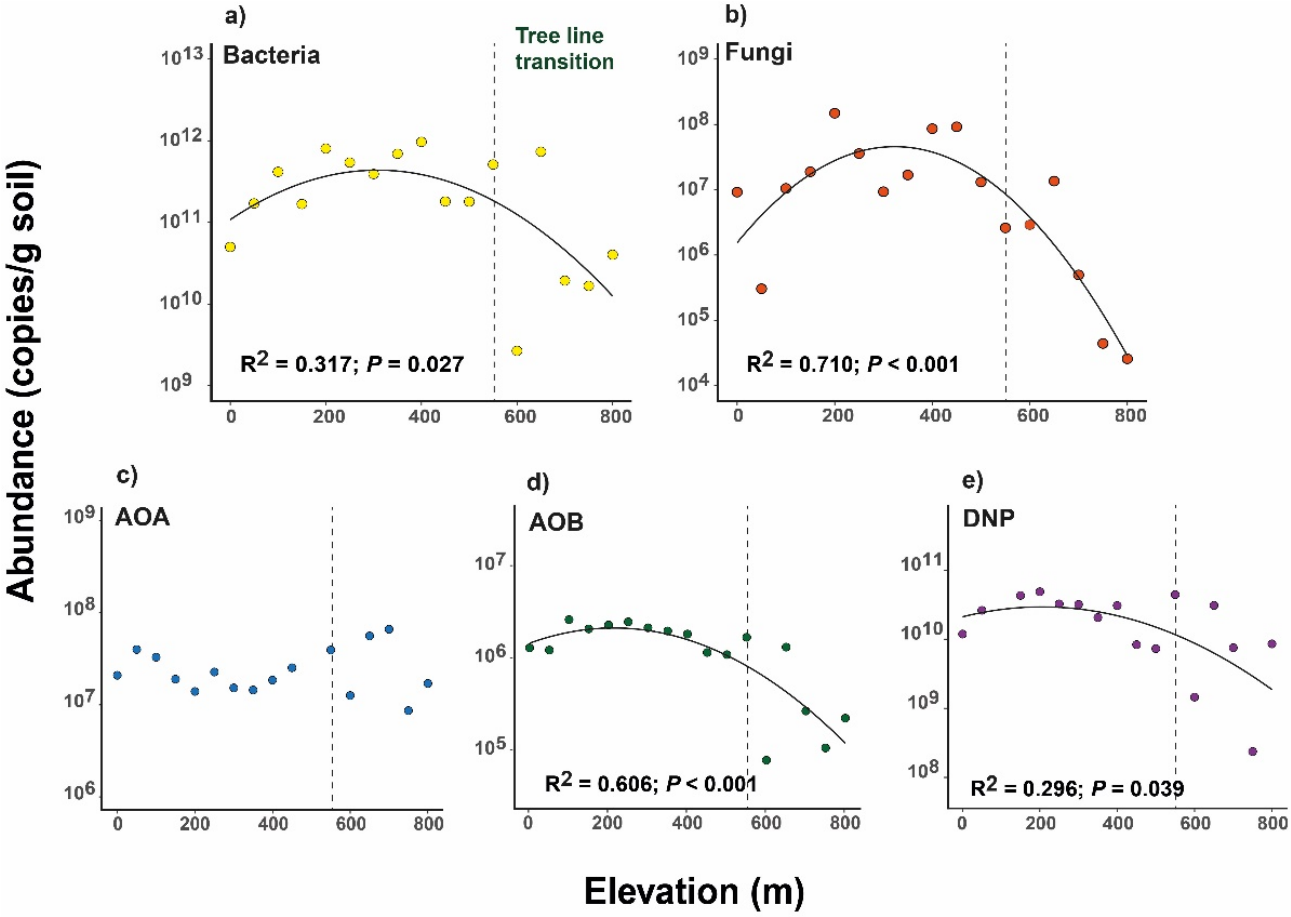


Figure 3: Taxonomic and functional abundance obtained by qPCR of fungal ITS and bacterial 16S amplicons and T-RFLP functional *amoA* and *nosZ* groups along the studied elevational gradient. Model fit statistics and AICc values describing the relationship between elevation and the abundance of taxonomic and functional groups are available in Table S2. AOA: ammonia oxidizing archaea; AOB: ammonia oxidizing bacteria; DNP: denitrifying prokaryotes.

Ecological drivers of taxonomic and functional networks

We found that soil microbial taxa grouped into three major taxonomic clusters (Fig. 5a). All clusters contained fungal and bacterial taxa strongly co-occurring with one another. Two clusters (#0 and #1) were related to elevation, yet in an opposite way (Fig. 5b; Table S3). While the relative abundance of Cluster#0 peaked at intermediate elevation, Cluster#1 showed its highest abundance at tundra sites. Cluster#3 was not significantly related to elevation (Fig. 5b; Table S3). Random Forest analysis showed that plant attributes (richness and productivity) and temperature were the most important predictors of Cluster#0 and Cluster#1, followed by habitat change and soil C content. Despite the absence of an elevational trend, Random Forest analysis identified soil C:N ratio as the most important predictor of Cluster#2.

Table 1. Summary table including the best fitting shapes (linear or quadratic relationship) for the regressions between microbial attributes and their main predictors determined by Random Forest analysis (excluding the categorical habitat change predictor). ●: positive linear; ●: negative linear; ●: concave unimodal; ●: convex unimodal functions. See Tables S2-S5 for actual models. C: soil carbon content; C:N: soil Carbon:Nitrogen ratio; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness; Tmin: average of Minimum daily temperatures; AOA: ammonia oxidizing archaea; AOB: ammonia oxidizing bacteria; DP: denitrifying prokaryotes.

	C	C:N	WHC	NPP	pH	PR	T_{min}
Fungal richness	●		●	●		●	●
Fungal abundance	●	●	●	●		●	
Bacterial richness				●		●	●
Bacterial abundance		●				●	
AOA richness				●			
AOB abundance	●		●	●		●	●
DP richness				●			●
Ecological Cluster#0	●	●	●	●		●	●
Ecological Cluster#1	●		●	●		●	●
Ecological Cluster#2		●			●		●
Animal pathogen fungi			●	●	●		●
Ectomycorrhizal fungi		●		●		●	●
Endophyte fungi	●		●	●		●	●
Ericoid mycorrhizal fungi					●		●
Fungal parasite fungi					●		●
Lichenized fungi				●		●	●

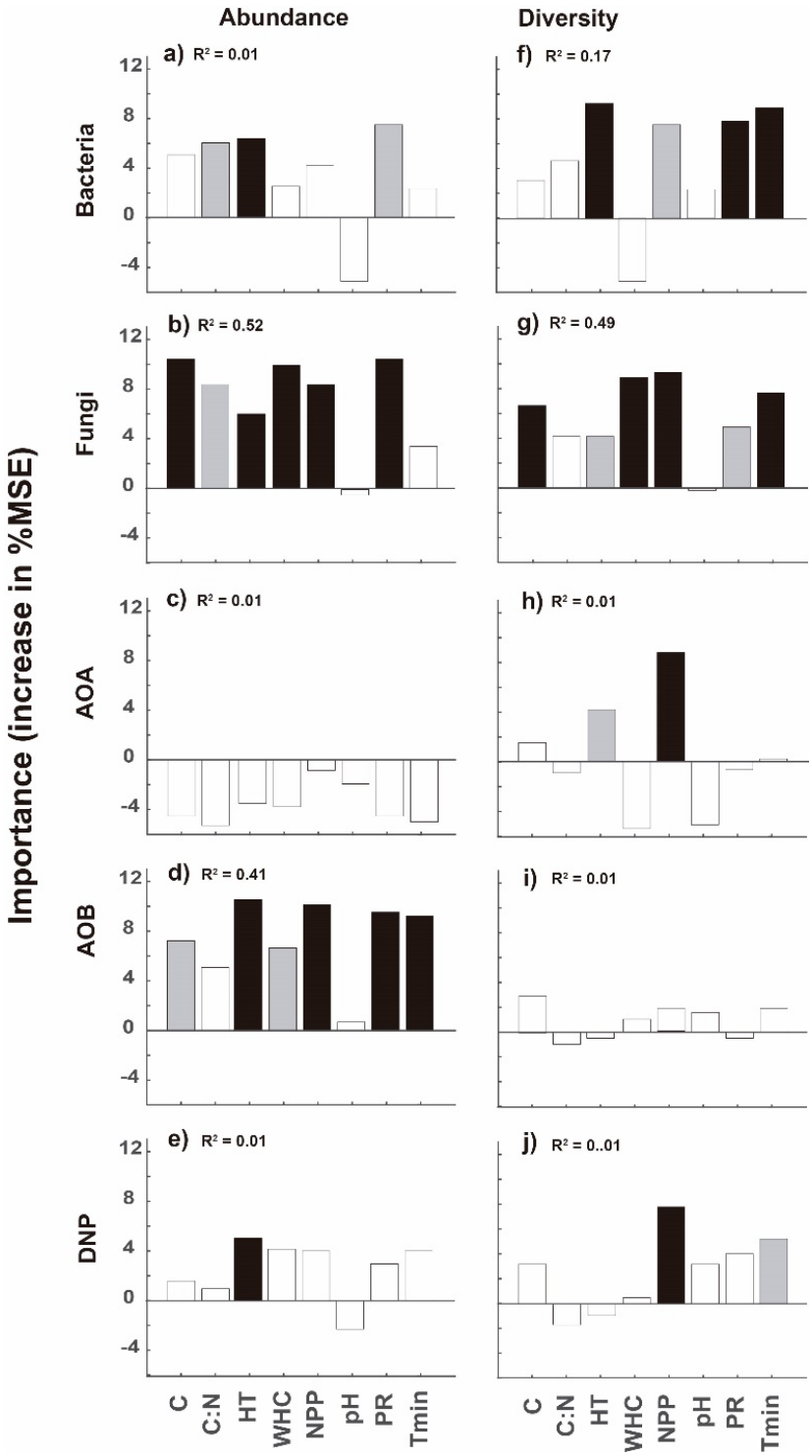


Figure 4: Random forest mean predictor importance (% of increase of mean square error) of environmental variables as drivers of diversity and abundance for soil bacteria, fungi and functional groups. AOA: ammonia oxidizing archaea; AOB: ammonia oxidizing bacteria; DNP: denitrifying prokaryotes; C: soil total C; C:N: soil C:N ratio, HC: habitat change; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness, Tmin: average of minimum daily temperatures.

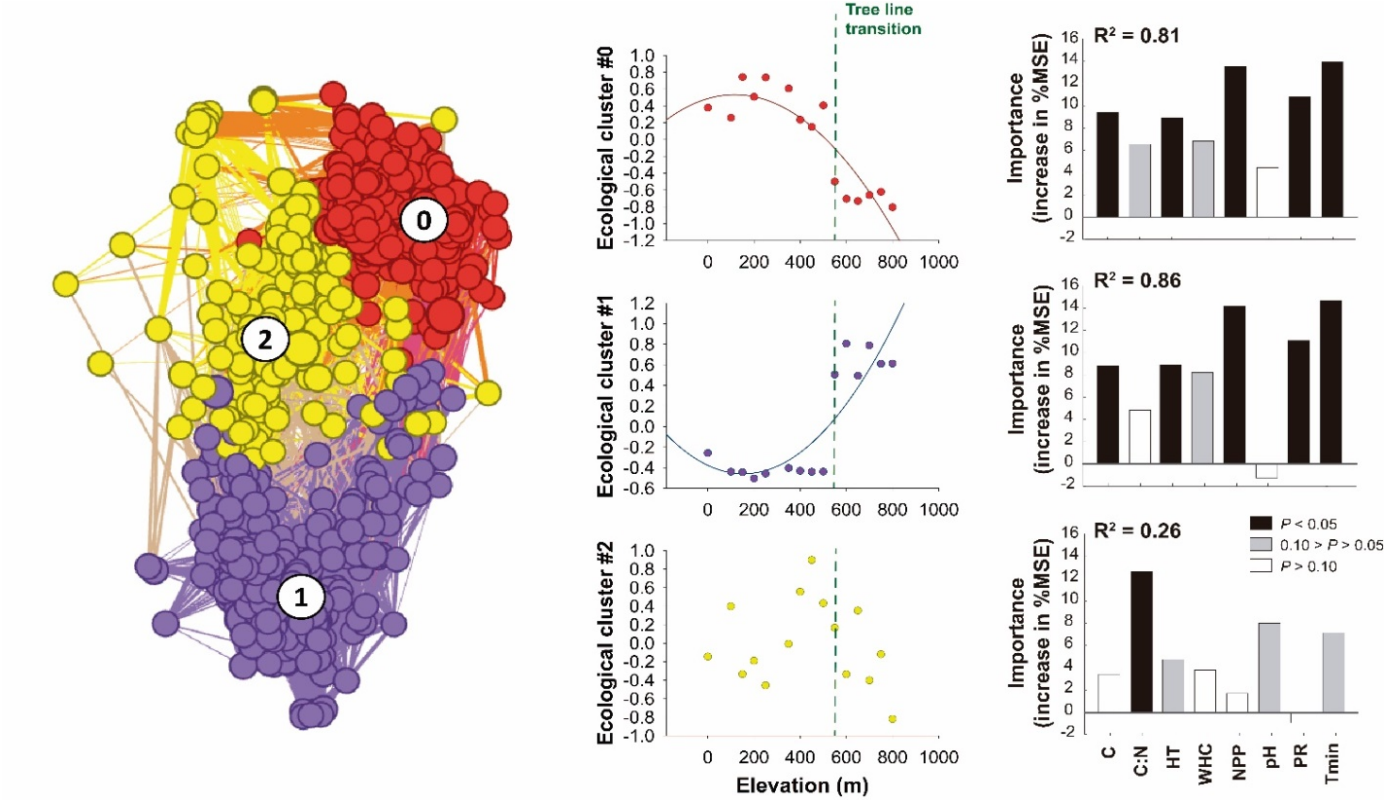


Figure 5: Co-occurrence network of soil microorganisms. (a) Network diagram with nodes (taxa of bacteria and fungi) colored by each of the major three identified clusters along the elevational gradient. (b) Relationship between the relative abundance of each soil cluster and elevation. (c) Random Forest mean predictor importance (% of increase of mean square error) of environmental variables as drivers of three clusters identified. Model fit statistics and AICc values describing the relationship between elevation and the relative abundance of Clusters#1-3 are available in Table S3.

Major microbial functional groups (AOA, AOB and DNP) assembled into five major functional clusters (Fig. S1a), comprising functional phylotypes strongly co-occurring with one another. Among these clusters, only Cluster#1 was related to elevation (Fig. S1b; Table S3). Any DNP phylotype was included in functional clusters #0, #2 and #3, with AOB phylotypes being dominant in 4 out of 5 functional clusters (i.e. #0, #1, #3 and #4). In this case, Random Forest analysis was not able to determine any significant predictor for any functional cluster (Fig. S2).

Ecological drivers of functional fungal groups

Across the entire fungal dataset, functional life styles were mostly symbiotic (mycorrhizal, endophyte or lichenized). Ectomycorrhizal taxa were by far the most abundant functional group along the elevational gradient studied. This group was especially abundant under forests, peaking at intermediate elevations (Fig 6b; Table S4). Similarly, ericoid mycorrhizal taxa occurred mostly under forest canopy and almost disappear from tundra soils (Fig. 6d; Table S4). Conversely, the relative abundance of functional groups like endophytes and lichenized fungi mostly increased at tundra sites (Fig. 6cf; Table S4). Random Forest analysis consistently identified Tmin as predictor for the abovementioned functional fungal groups, as well as for animal pathogens and fungal parasites (Fig. S3). Plant attributes (net primary productivity and plant richness) and habitat change were important predictors for symbiotic groups (i.e. ectomycorrhizal, endophytes, ericoid mycorrhizal, and lichenized; Fig. S3; Table S4). Finally, pH was an important predictor for ericoid mycorrhizal, animal pathogens and fungal parasites (Fig. S3; Table S4).

Changes in microbial community composition with elevation

Most of the fungal phylotypes were classified as Ascomycota (52%), Basidiomycota (24%) and Zygomycota (3%), while bacterial phylotypes belonged to Proteobacteria [30% (α-Proteobacteria (12%); β-Proteobacteria (4%), γ-Proteobacteria (5%) and δ-Protebacteria (7%)], Planctomycetes (12%), Acidobacteria (9%), Bacteroidetes (8%), Actinobacteria (8%) and Verrocumicrobia (6%) (Fig. S4). Nevertheless, the relative abundance of major phyla and the taxonomic and functional community composition at the phylotype level (measured as the first axis of a NMDS) largely differed between elevations (Fig. S5). Soils under forest canopies (0-550 m) were dominated by Basidiomycota, with Ascomycota as the second most abundant phylum (Fig. S4a). In soils sampled from above the tree line level (550 m), both phyla

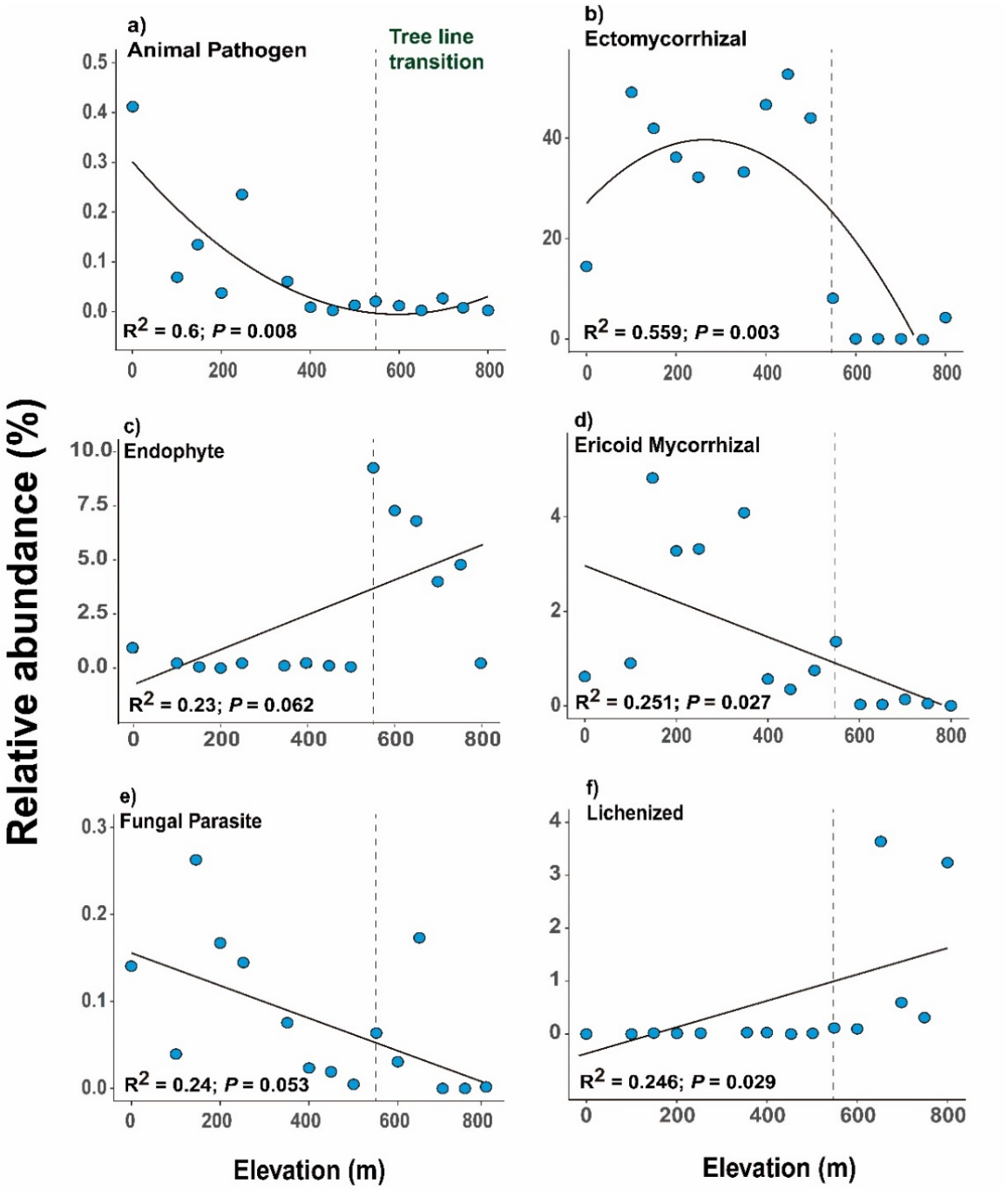


Figure 6: Relative abundance of major fungal functional groups (identified from fungal taxa across the entire ITS dataset with the online application FUNGuild) along the elevational gradient studied. Model fit statistics and AICc values describing the relationship between elevation and the relative abundance of functional groups are available in Table S4.

switched positions, with Ascomycota as the dominant fungal taxa. Zygomycota was the third most relatively abundant phylum throughout the elevation gradient evaluated (except at 0 and 800 m). Chytridiomycota was a minority group but its relative abundance increased at higher elevations.

The bacterial community contained many more phylotypes than the fungal one, and was dominated by Proteobacteria, Acidobacteria and Actinobacteria; these taxa accounted for up to 70% of relative abundance at the forest (Fig. S4b). The dominance of Proteobacteria and Acidobacteria decreased and increased, respectively, at the tundra, while groups such as Chloroflexi and AD3 became particularly abundant at this elevation level. Proteobacteria were always the most relatively abundant phylum throughout all elevations (except at 650 m). α-Proteobacteria had the highest relative abundance within this phylum, albeit it decreased at elevations above treeline ecotone, while β- and γ-Proteobacteria remained almost constant along the elevational gradient evaluated. δ-Proteobacteria had the lowest relative abundance within this phylum, with a steady low presence that, however, dramatically increased at the highest elevation to become the most abundant Proteobacteria class (36% of total Proteobacteria relative abundance). Actinobacteria, Bacteroidetes and Planctomycees followed a similar decreasing pattern with elevation, but Acidobaceria followed an opposite trend. Chloroflexi, Cyanobacteria and AD3 had higher relative abundance in tundra than in forest soils.

In general, AOA, AOB and DNP communities did not abruptly change with the forest to tundra transition (Fig. S6), as observed for bacterial and fungal communities. However, the dominance of major phylotypes in the DNP community did vary with elevation, specially 106 (becoming the dominant phylotype in tundra soils), 54 (drastically reduced above tree line) and 50 BP fragments (practically disappearing above tree line).

Relationship between taxonomic and functional microbial diversity and abundance with soil processes.

Correlations of microbial abundance and diversity with soil functions are shown in Table 2. Several significant relations were observed between soil functions and microbial richness or abundance. Soil nutrients were mostly related to fungal abundance, bacterial diversity and AOB abundance. Fungal diversity was negatively related to most soil variables, specially ammonium and potential mineralization, while fungal abundance positively related to several "functional surrogates" (i.e. nitrate and potential mineralization). Bacterial

Table 2: Spearman rank correlations of microbial 16S and ITS and functional group species diversity and abundance with soil functions. N: soil total N; C: soil total C; Corg: soil organic carbon; DON: dissolved organic N; N-BM: microbial biomass N; NIP: potential nitrification rate; MIN: potential mineralization rate; AIP: available inorganic P; AG: α-Glucosidase; BG: β-Glucosidase; CB: β-D-cellobiosidase; LAP: L-Leucine- aminopeptidase; NAG: N-acetyl-β-glucosaminidase; PHOS: Phosphatase; XYL: β-Xylosidase. a = P-valor < 0.1, * = P-valor < 0.05; ** = P-valor < 0.01.

	Fungi		Bacteria		AOA		AOB		DP	
	Div.	Ab.	Div.	Ab.	Div.	Ab.	Div.	Ab.	Div.	Ab.
pH	-0.322	-0.047	0.388	-0.072	-0,176	-0.085	-0,235	0.453	0,246	0.346
EC	-0.710**	0.517*	0.431	0.456	-0,214	0.047	0,432	0.748**	0,309	0.541*
WHC	-0.480	0.475	0.177	0.486*	-0,071	0.247	0,403	0.664**	0,035	0.514*
N	-0.354	0.520*	0.113	0.461a	-0,134	0.203	0,533a	0.632**	-0,121	0.388
C	-0.341	0.537*	0.056	0.522*	-0,103	0.191	0,611*	0.637**	-0,033	0.429a
Corg	-0.371	0.596*	0.181	0.588*	0,052	0.203	0,625*	0.681**	-0,004	0.550*
AN	-0.459a	0.493*	0.740**	0.380	0,138	-0.215	0,088	0.392	-0,091	0.476
NH4	-0.650*	0.582*	0.611*	0.489a	-0,059	-0.121	0,178	0.771**	0,192	0.496a
NO3	-0.398	0.657**	0.694**	0.404	0,049	-0.109	0,386	0.419a	-0,255	0.471a
DON	0.343	0.000	0.475a	0.096	0,509a	0.125	0,466	-0.014	-0,201	-0.054
N-BM	-0.547*	0.554*	0.243	0.395	-0,186	0.156	0,684*	0.507*	0,523*	0.441
C:N	-0.385	0.583*	0.135	0.635**	-0,054	0.147	0,467	0.544*	0,004	0.415
NIP	0.273	0.150	0.211	-0.089	0,460a	-0.099	-0,041	0.018	-0,043	-0.046
MIN	-0.705**	0.696**	0.632**	0.426a	-0,225	-0.100	0,435	0.600*	0,097	0.429a
AIP	-0.546*	0.209	0.557*	0.278	-0,252	-0.125	-0,407	0.422a	0,186	0.450a
AG	-0.591*	0.120	-0.020	-0.027	-0,264	0.235	0,618**	0.034	0,594*	0.059
BG	-0.324	0.182	-0.312	-0.188	-0,442	0.354	0,187	-0.038	0,430	-0.050
CB	-0.280	0.011	-0.489a	-0.107	0,042	0.125	0,489	-0.143	0,224	-0.156
LAP	0.042	-0.179	0.042	-0.034	-0,153	-0.209	0,228	-0.049	0,286	-0.074
NAG	0.319	-0.225	-0.392	-0.358	0,405	-0.012	-0,246	-0.664**	-0,154	-0.591*
PHOS	0.121	-0.091	-0.348	-0.358	0,507*	-0.141	-0,126	-0.615**	-0,100	-0.571*
XYL	-0.497	0.139	0.150	0.125	-0,161	0.138	0,738*	0.082	0,391	0.032

diversity also positively related to N forms and P availability, as well as potential mineralization. However, bacterial abundance was mostly linked to soil C content and C:N ratio. Regarding functional groups, AOB and DP abundances showed the most important relations.

AOB abundance was highly related to N and C variables (i.e. soil total N and ammonium, and soil total C) and DNP abundance correlated to N forms (ammonium and nitrate) and soil C. AOA and DNP diversity did not relate to most soil functions. Regarding soil enzymatic activities, most of them showed a general weak relation to microbial abundance and diversity. AG was negatively related to fungal diversity but positively to AOB and DNP diversity. NAG was negatively related to AOB and DNP abundances, similarly to PHOS, but also positively related to AOA diversity.

Discussion

Our study provides novel evidence that habitat (forest/tundra transition), net primary productivity and plant richness are the most consistent predictors for variation in the diversity, abundance and co-occurrence network of soil microbial communities in the sub-Antarctic region, which remains largely understudied so far. Interestingly, elevational patterns in soil microbial diversity in this region varied with the taxonomic and functional group considered. For example, the diversity of fungi tended to increase with elevation, while the opposite pattern was found for the diversity of soil bacteria and bacterial nitrifiers. However, we also found that changes in the abundance of these organisms were quite consistent across taxonomic and functional groups, as most of them followed a hump-shaped relationship with elevation. Additionally, we identified ecological clusters of taxonomic and functional soil microbes, and observed that particular clusters followed opposite elevational trends. We further identified the dominant functional fungal life styles along the elevational gradient. Fungal life styles shifted with forest/tundra transition from mycorrhizal, animal pathogens and fungal parasites to lichenized and endophyte fungi.

Elevational trends for soil microbial diversity and abundance

Our results show that microbial diversity (taxonomic and functional) deeply changed with elevation in Navarino Island. These results partially support our first hypothesis (elevational patterns will led to drastic changes in the diversity of microbial communities, decreasing with elevation), as only bacterial and AOB richness decreased with elevation. We observed a strong increase in fungal diversity with elevation, a result seldom reported in studies

conducted along elevational gradients (Bahram et al., 2012; Looby, Maltz, & Treseder, 2016; Yang, Lü, Jiang, Shi, & Liu, 2017). However, this pattern was highly taxon-specific. For instance, Ascomycota diversity increased at high elevations, while Basidiomycota sharply decreased above tree line (Fig. S7). These divergent trends may suggest a high fungal dependence on plant species turnover (and subsequent habitat transition). This interpretation is supported by the changes in fungal life-style observed, which are further discussed below. Basidiomycota has been reported to be a dominant phylum in fungal communities from *Nothofagus* forest soils (Nouhra *et al.*, 2013). For instance, numerous Basidiomycota species from the genera *Cortinarius*, *Inocybe*, *Rickenella* and *Mortierella* – important components in rhizosphere of *Nothofagus* forests as saprophytes or mycorrhizal (Dickie *et al.*, 2009; Tedersoo *et al.*, 2009; Nouhra *et al.*, 2013)– appeared almost exclusively in *Nothofagus* dominated soils. This is supported by the analysis of fungal major life-styles, with ectomycorrhizal and ericoid mycorrhizal groups dominating in mid-elevation sites. The other fungal phyla (Chytridiomycota, Glomeromycota and Zygomycota) did not display any clear elevational trend (Fig. S7), and accounted for a small part of the total fungal diversity observed. Freeman et al. (2009) reported high diversity values for Chytrids in alpine soils, but the low elevation of our alpine soils vs. that reported in that study (800 m vs. more than 5000 m) may explain this discrepancy.

Unlike soil fungal diversity, the diversity soil bacteria was negatively correlated to elevation. Our results are consistent with previously reported trends for bacteria at different taxonomic levels (Bryant *et al.*, 2008; Singh *et al.*, 2012; Delgado-Baquerizo *et al.*, 2016c; Peay *et al.*, 2017). More precisely, we observed that bacterial diversity followed the same elevational trend (hump-shaped) reported for plants and animals in Navarino Island (Contador *et al.*, 2015; Molina *et al.*, 2016). This contrasts with recent studies reporting that decoupled trends may exist in the diversity patterns of microbes and those of plants and animals along elevation gradients from tropical and temperate ecosystems (Fierer et al., 2011; Shen et al., 2014). Nonetheless, the observed trend was again not consistent when looking at the phylum level (Figs. S8-S9), supporting the frequently accepted statement of taxon-dependent trends in bacterial diversity along elevational gradients (Looby et al., 2016; Shen et al., 2013; Wang et al., 2015). For example, while richness of Actinobacteria, Bacteroidetes, Planctomycetes, Proteobacteria and Verrucomicrobia –dominant taxa in our gradient– peaked at low-mid elevations (Figs. S7-S8), that of other important phyla - Armatinomonadetes, Chloroflexi and Cyanobacteria- peaked in the alpine sites. Some of

these groups, such as Chloroflexi and, particularly, Cyanobacteria, are very important in alpine environments (Costello & Schmidt, 2006; Zakhia *et al.*, 2008), increasing N availability and promoting ecosystem development (Duc *et al.*, 2009; Bajerski & Wagner, 2013). Moreover, the higher richness of these groups at high elevations can be explained by their high resistance to harsh conditions (i.e. survival to desiccation), thriving under the most arid and resource-poor environments (Maestre *et al.*, 2015; Delgado-Baquerizo *et al.*, 2016c). In addition, both Chloroflexi and Cyanobacteria have protection pigments against UV light, which is expected to be the highest at the top of our elevational gradient, especially in this ozone-depleted region (Rousseaux *et al.*, 2001).

The diversity of functional microbes involved in N-cycling (AOA, AOB and DNP) varied along the gradient depending on the group considered, supporting our third hypothesis. Diversity of archaeal nitrifiers (AOA) –usually referred as cold-adapted extremophiles (Cavicchioli, 2006)– seemed to be slightly higher in alpine locations of this sub-Antarctic ecosystem, although a clear elevational patter was not detected. Conversely, AOB are known to prefer nutrient-rich environments (e.g. high soil organic C –a common proxy of organic matter; Delgado-Baquerizo *et al.*, 2016c). Therefore, reductions in resource availability linked to reduced plant cover with elevation might largely influence the diversity of AOB organisms.

The abundance of microbial groups followed a hump-shaped elevational trend in four out of five cases. This again partially supports our first hypothesis of elevation negatively impacting microbial attributes. The drastic decrease in fungal and bacterial abundance with elevation is congruent with a progressive decrease in organic matter (soil organic C) content in alpine soils. Regarding functional groups, our results fit with the general finding of archaea dominating the abundance of *amoA* genes in most ecosystems (Leininger *et al.*, 2006; Nicol *et al.*, 2008; Zhang *et al.*, 2009). However, a clear trend for AOA could not be detected. Our results are partly coincident with those describing an abrupt decrease of *amoA* abundance with elevation, as found by Zhang et al. (2009) in the Everest.

Ecological drivers of taxonomic and functional networks.

Our network analysis provided clear evidence of a strong niche-partitioning of co-occurring soil microbes, mostly mediated by drastic habitat changes along the elevational gradient studied. For example, Cluster#0 was drastically reduced at high elevation but peaked at low-mid elevation, where trees dominate the landscape. This cluster contains members of

bacterial and fungal groups such as Bradyrhizobiaceae and Cortinariaceae, which include some rhizosphere microorganisms (symbiotic or parasitic) groups (Tedersoo *et al.*, 2009; Buée *et al.*, 2014; Hardoim *et al.*, 2015). This cluster also includes species involved in plant growth promotion and protection against pathogens (i.e. *Variovorax paradoxus* and *Burkholderia bryophila*; Han et al., 2011; Vandamme et al., 2007). We acknowledge that without including other eukarya groups (e.g. protozoa or algae), establishing parasite-host or predator-prey relationships among microbes is not possible. However, our results are coincident with recent studies (Shi *et al.*, 2016) pointing to *Rhizobium,* Burkholderiales and Pseudomonadales as important taxa characterizing microbial clusters in plant covered soils. The order Rhizobiales is negatively affected by low temperature (Oliverio *et al.*, 2017), supporting its inclusion in cluster#0 (positively related to temperature). Furthermore, it has been suggested that some of these groups are involved in microbial interactions coordinating soil processes (e.g. N cycling) via mechanisms such as quorum sensing (Deangelis *et al.*, 2008; Shi *et al.*, 2016). On the contrary, cluster#1 peaked at high elevations, just immediately after forest replacement by sub-Antarctic tundra. This cluster contains typical alpine taxa such as lichenized fungi (e.g. *Ochrolechia*) and endophytes involved in plant stress reduction under harsh environments (e.g. *Phialocephala fortinii*; Jumpponen et al. 1998). The genus *Capronia* –described as plant endophyte but also lichenicolous (Etayo *et al.*, 2013)–, was especially abundant in (and exclusive to) this cluster.

Ecological drivers of microbial diversity and abundance

We found that plant related attributes such as habitat change, plant richness and productivity were the major ecological predictors explaining the observed elevational patterns in microbial diversity and abundance. Plant attributes are key components of aboveground-belowground interactions (Bardgett, 2010). For example, plant diversity is well-known to benefit microbial community via increased resource availability (diverse debris sources and exudates) and physical (microclimatic variability and habitat complexity) heterogeneity associated to plant diversity (Waldrop *et al.*, 2006; Prober *et al.*, 2015). Recent studies have shown that plant richness is associated (often positively) to microbial community richness and functional diversity (De Deyn *et al.*, 2011; Lamb *et al.*, 2011; Hiiesalu *et al.*, 2014; Chen *et al.*, 2017; Yang *et al.*, 2017). For example, Hiiesalu *et al.* (2014) found that plant richness is positively associated to arbuscular mycorrhizal fungi richness in a grassland from Canada. Moreover, plant richness and NPP are directly related to soil organic carbon quality and quantity, which is known to directly impact on both microbial abundance and community

composition (Lange *et al.*, 2014, 2015; Maestre *et al.*, 2015). In addition, higher plant species richness has been related to higher microbial biomass and abundance (Zak *et al.*, 2003), mostly via higher plant productivity associated with greater plant diversity (Tilman *et al.*, 2001, 2012). The observed habitat transition led to a drastic reduction in plant species richness and, therefore, in primary productivity and ultimately in soil organic matter (Fig. S10). This may explain the predominant role of habitat change as predictor of microbial attributes. We found that soil total C and the C:N ratio were also important predictors for microbial diversity and abundance, which agrees with the view that the C:N ratio is a major factor in determining soil microbial community structure (Wan *et al.*, 2014). Minimum temperature was also an important variable helping to explain both fungal and bacterial richness along the elevational gradient studied. Temperature is usually positively correlated with microbial diversity (Zhou *et al.*, 2016) but minimum temperature has been previously related to higher probability of presence of some fungal groups (Claridge *et al.*, 2000). Temperature exerts a major role in plant attributes, especially forest-tundra transition. Thus, the role of minimum temperature could be both directly and indirectly (via conditioning food input to soil) driving microbial richness along the gradient. Random forest analysis discarded pH as an important predictor for both diversity and abundance data in our gradient (Fig 4). However, pH, usually recognized as an important factor shaping bacterial communities (Bryant et al., 2008; Chu et al., 2010; Shen et al., 2013; Siles & Margesin, 2016; Wang et al., 2015), was an important predictor of fungal life styles (e.g. animal pathogen or ericoid mycorrhizal, Fig. S3). Interestingly, our elevational gradient falls into the range of pH (3.66-5.38) for which diversity of bacteria is expected to change profoundly with small changes of pH (Lauber *et al.*, 2009). However, the pH range of most of our gradient (88% of plots) falls between 4 and 5 with no difference between lower and upper soils (4.78 *vs.* 4.75, respectively). Thus, the detected pH range does not represent effective elevational changes in our study area, and this may explain the lack of importance of pH as a predictor of changes in microbial communities with elevation.

The diversity of functional groups involved in N cycling was less related to the environmental variables evaluated than that of total bacteria and fungi (Fig. 4). The richness of denitrifiers was negatively linked to plant NPP ratio and Tmin, but not to pH as is usually assumed for denitrifiers (Prosser & Nicol, 2012). AOA abundance and AOB diversity were also not predicted by random forest analysis. This suggests that ammonia oxidizers may display more complex interactions with other variables not explored in our study.

Elevational changes in microbial community composition and fungal life-styles

The structure of fungal and bacterial communities was, as described for previous microbial attributes, highly sensitive to elevation and intimately linked to habitat change along the elevational gradient studied (Fig. S4-S5). Forest habitat soils were dominated by bacterial and fungal groups tightly related to vegetation and high soil C content. It is well known how elevation influences plant species diversity and productivity, which in turn conditions major microbial lineages linked to them, such as symbionts, parasites and saprophytes (Fig. 6). For example, Proteobacteria –the dominant phylum in forested soils (0-550 m)– is known to establish plant symbiotic (e.g. *Rhizobium*) and pathogenic relationships (Hardoim *et al.*, 2015). This phylum was also found dominant under *Nothofagus antarctica* and *N. pumilio* soils in Tierra del Fuego (Chile; Fernández-Martínez *et al.*, 2016, 2017). Conversely, characteristic phyla from mineral soils, such as *Chloroflexi* and *Cyanobacteria*, gained importance thriving in tundra soils. Extended discussion on the major changes in bacterial community composition with elevation observed in this study is available in Appendix S2.

The most drastic changes in community composition were observed in the fungal community (Fig. S4), with a clear habitat segregation in the dominance of Basidiomycota and Ascomycota. Basidiomycota –a typical dominant group in forested soils (Buée *et al.*, 2014)–, was the major component of the ectomycorrhizal guild. This symbiotic lifestyle is of great benefit for the development of plants (Hardoim *et al.*, 2015), and was by far the most prominent functional fungal group bellow timberline (Fig. 6). The higher proportion of animal pathogens at low elevations may be explained by the greater animal presence (both cattle and feral) in lowlands of Navarino Island (Molina *et al.*, 2016). Conversely, tundra soils above 550 m were dominated by Ascomycota, mostly characterized as endophytic, lichenized and saprobes. This phylum is a typical dominant phylum in nutrient-poor ecosystems (Schadt, 2003; Bates *et al.*, 2012; Maestre *et al.*, 2015). Little is known of the role of endophytes in extreme environments, although a protective role against herbivores, parasites and environmental stress is hypothesized (Bråthen *et al.*, 2015). Unfortunately, the great proportion of unclassified taxa in this harsh environment prevents a more detailed explanation of the functional structure of microbial communities at the tundra.

Relationship between taxonomic and functional microbial diversity and abundance with soil processes

We found multiple relationships between soil taxonomic and functional diversity and abundance with evaluated soil functions. Fungal diversity, which increased with elevation, was negatively related to all measured soil functions, specially NH_4^+ and potential mineralization. The observed fungal diversity trend may reflect a progressive gain in fungal species preferring cold environments and involved in recalcitrant compounds degradation in tundra soils. The hump-shaped trend of fungal abundance prevents similar negative correlations and coincide with better nutrient availability conditions (NO_3^-, AN, C:N, and C –a common surrogate of organic matter) at mid elevations. This may be explained by a higher presence of nutrient cycling groups (saprophyte and mycorrhizal) at these locations. Bacterial diversity was correlated with N functions (N forms and mineralization), while bacterial abundance was related to C:N ratio. AOA and AOB diversity did not correlate with most soil functions. AOB abundance, but not AOA, was positively correlated with total soil N and NH_4^+, as well as NO_3^-, and total and organic C, which partly reinforces the idea that AOB organisms are copiotrophic communities, while AOA are not affected by nutrient availability (Zhang *et al.* 2009; Delgado-Baquerizo *et al.* 2016b)

Regarding enzyme activity, we surprisingly observed a general lack of significant correlations between selected enzymes and taxonomic and functional diversity and abundance. Both fungi and bacteria are known to produce extracellular enzymes for organic matter decomposition and nutrient mobilization (Trivedi *et al.*, 2016). It has even been proposed that fungi exert more control than bacteria over enzyme production, especially for cellulose and lignin degradation (Schneider *et al.* 2012; Baker and Allison 2017). However, we found that fungal diversity was just correlated to sugar degradation (AG) and bacterial diversity to cellulose degradation (CB). Both fungal and bacterial abundances did not show significant correlations. Functional groups AOB and DP abundances were highly correlated to chitin degradation (NAG) and phosphorus mobilization (PHOS). This can indicate high resource allocation by nitrifiers and denitrifiers to enzyme synthesis to prevent nitrogen transformation limitation by low phosphorus availability (Mehnaz & Dijkstra, 2016).

Conclusions

Here we provide clear evidence that plant community attributes such as species richness and productivity, together with habitat change, are consistent predictors of microbial diversity, abundance and co-occurrence network in the studied sub-Antarctic region. Despite observed elevational patterns in microbial diversity varied with taxon and/or functional group considered, the relationship between microbial abundance and elevation was largely

predictable and followed a hump-shaped relationship for most evaluated taxonomic and functional groups. Moreover, we found that the transition between forest and tundra triggers substantial changes in the life-styles of fungal communities leading to changes from mycorrhizal-dominated to endophyte-dominated communities. Together, our results offer insights on the drivers of microbial attributes across elevational gradients in a sub-Antarctic region, which are of paramount importance to improve our capacity to understand the impacts of ongoing climate change on the structure and functioning of this unique ecosystem.

Supplementary material

Appendix 1: *Supplementary methods.*

Soil function analysis

Soil total N was measured with a CN analyzer (Leco CHN628 Series; Leco Corporation, St Joseph, MI, USA). Soil total organic C was determined by colorimetry after oxidation with a mixture of potassium dichromate and sulfuric acid as described in Anderson and Ingram (1990). Ammonium and nitrate (mineral N) and total available N were analyzed by colorimetry from K_2SO_4 0.5 M soil extracts using a 1 : 5 soil/extract ratio as described in the study by Delgado-Baquerizo *et al.* (2011). The concentration of DON was calculated as the difference between total and mineral N in the soil extracts. Microbial biomass N was determined using the fumigation–extraction method (Brookes *et al.*, 1985). Soil samples were fumigated with chloroform for 5 days. Non-fumigated replicates were used as controls. Fumigated and non-fumigated samples were extracted with K_2SO_4 0.5 M and total available N was determined as indicated above. Microbial biomass N was determined as the N difference between fumigated and non-fumigated samples. Potential net N mineralization and depolymerization rates were estimated following Allen et al. 1986. Soil samples were incubated in dark condition at 30 °C and 80 % of water holding capacity during 14 days. Initial and final soil subsamples were extracted with 0.5 M K_2SO_4, and analyzed for mineral and total N following the procedure described above. Potential net N mineralization and depolymerization rates were estimated as the difference between initial and final NO_3^--N, NH_4^+-N and DON concentrations, respectively. PO_4^{-3} was determined by colorimetry from a 0.5 M $NaHCO_3$ extraction (Bray & Kurtz, 1945).

Enzyme analysis

We measured the potential activity of seven hydrolytic soil enzymes involved in the degradation of common constituents of organic matter: α-Glucosidase (AG), β-Glucosidase (BG), β-D-cellobiosidase (CB), L-Leucine aminopeptidase (LAP), and N-acetyl-β-Glucosaminidase (NAG), Phosphatase (PHOS) and β-Xylosidase (XYL). Enzyme activities were measured using 4-methylumbelliferyl (MUB) substrate yielding the highly fluorescent cleavage products MUB upon hydrolysis. All the enzyme assays were set up in 96-well microplates following Bell *et al.* (2013). Fluorescence was measured using a microplate fluorometer (Synergy™ HTX Multi-Mode Microplate Reader, BioTek Instruments, Inc., USA). The activities were expressed as $nmol \cdot h^{-1} \cdot g^{-1}$ dry soil.

Appendix 2: *Elevational changes in microbial community composition, extended discussion.*

The structure of bacterial community reflected habitat changes along the elevational gradient studied. While Proteobacteria dominated organic forest soils, Acidobacteria were particularly abundant in mineral alpine soils. Proteobacteria –a dominant bacterial phyla in soils across the globe (Delgado-Baquerizo *et al.*, 2018a)– thrives in forest litter horizon with easily accessible carbon substrates (López-Mondéjar *et al.*, 2015). Besides, Proteobacteria is of paramount importance in the rhizosphere microbiome (Gschwendtner *et al.*, 2016). This explains the observed dominant position of this group in forested soils (0-550 m) with high organic C content. Conversely, Acidobacteria tend to gain relevance in tundra soils (Zinger *et al.*, 2009; Männistö *et al.*, 2013; Deng *et al.*, 2015), which are characterized by low pH and poor nutrient content. This phylum is also one of the most abundant soil bacterial phyla (Delgado-Baquerizo *et al.*, 2018a), and is considered an oligotrophic group despite its high heterogeneity and metabolic versatility (Lladó *et al.*, 2017). The most drastic change in relative abundance observed along the gradient was found for Chloroflexi and AD3 groups, which became particularly important at the tundra. Chloroflexi, a characteristic phylum from mineral soils, are adapted to the use of recalcitrant C substrates and inorganic nutrients (Lladó *et al.*, 2017). Finally, the progressive increase in the relative abundance of Cyanobacteria with elevation is a typical situation reported for this phylum (Fierer *et al.*, 2011; Janatková *et al.*, 2013).

Although the structure of bacterial communities showed evident habitat segregation, the structure of functional communities did not respond in a similar way (Fig. S6). Top AOA phylotypes maintained a dominant position, regardless habitat change across elevation. This

suggests that generalist AOA phylotypes dominate the community, which may confer a high resilience of the community to different climate change scenarios. Top AOB phylotypes did not show any clear trend, while the DNP community was highly sensitive to elevation and subsequent habitat turnover.

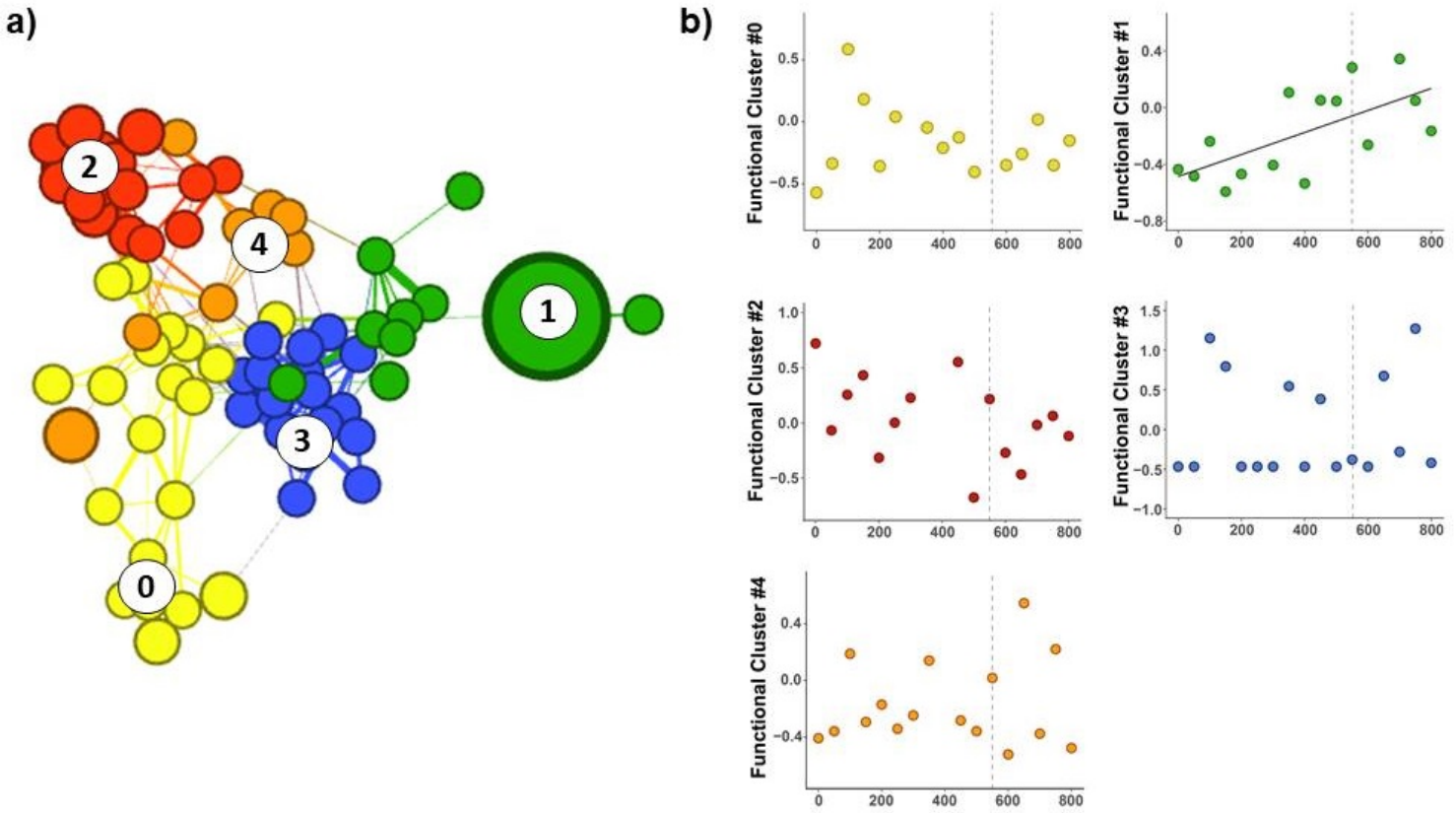


Figure S1: Co-occurrence network of soil functional phylotypes. (a) Network diagram with nodes (ammonia oxidizing bacteria and archaea and denitrifying prokaryotes) colored by each of the major five clusters identified along the elevational gradient. (b) Relationship between the relative abundance of each soil cluster and elevation. Model fit statistics and AICc values describing the relationship between elevation and the relative abundance of Clusters#0-4 are available in Table S3.

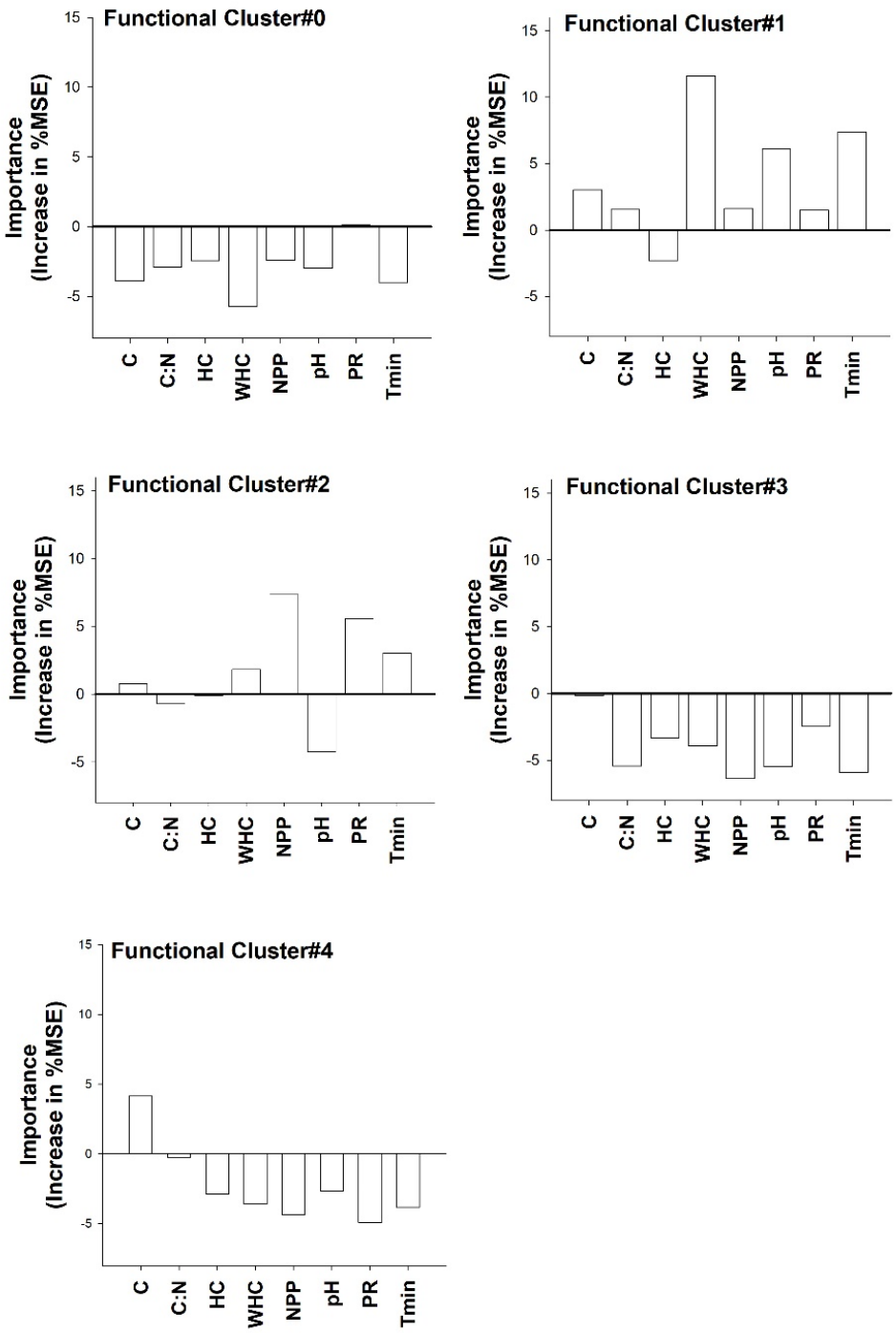


Figure S2: Random Forest mean predictor importance (% of increase of mean square error) of environmental variables as drivers of the five functional clusters identified. C: soil total C; C:N: soil C:N ratio, HC: habitat change; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness, Tmin: average of minimum daily temperatures.

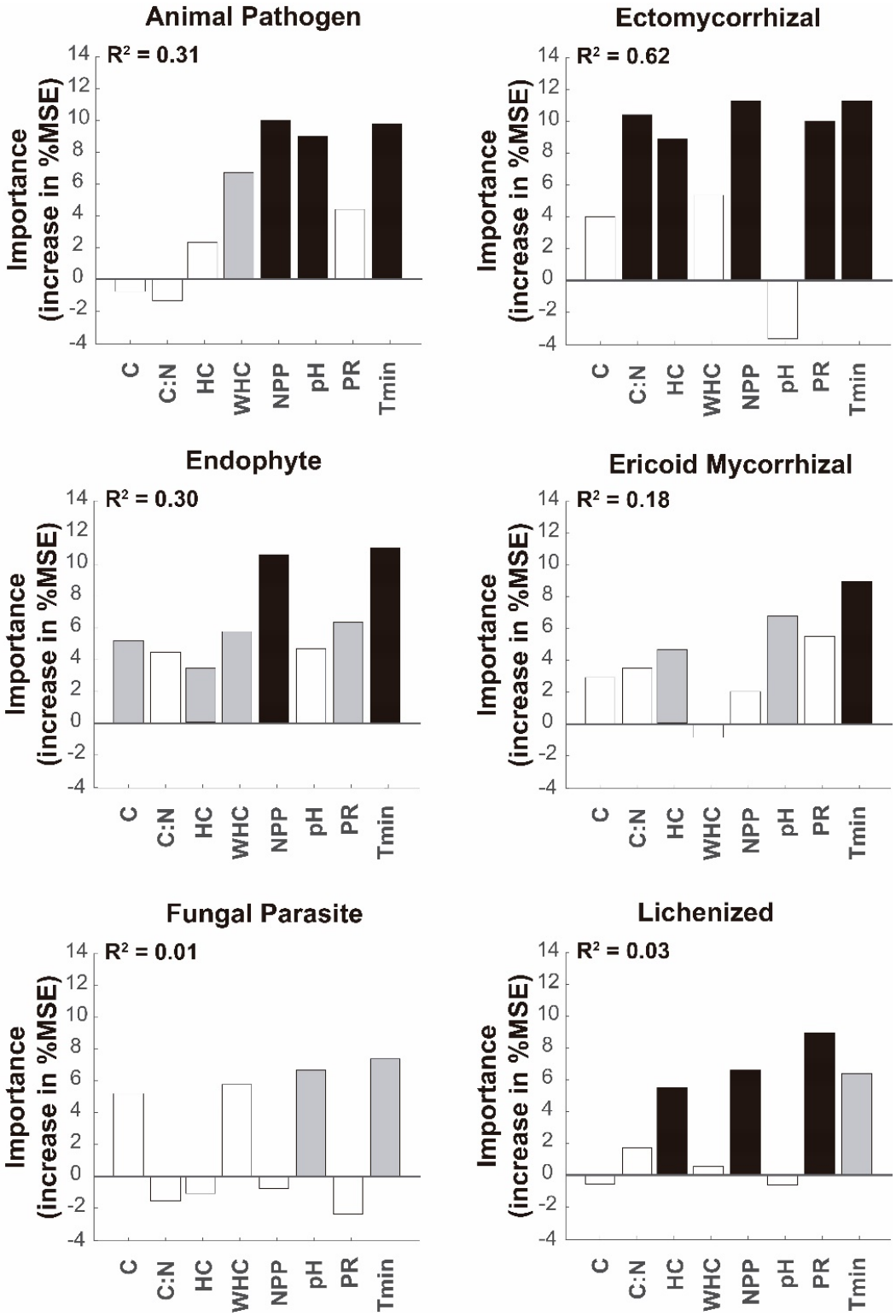


Figure S3: Random Forest mean predictor importance (% of increase of mean square error) of environmental variables as drivers of main fungal functional life-styles identified from fungal taxa across the entire ITS dataset with the online application FUNGuild. C: soil total C; C:N: soil C:N ratio, HC: habitat change; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness, Tmin: average of minimum daily temperatures.

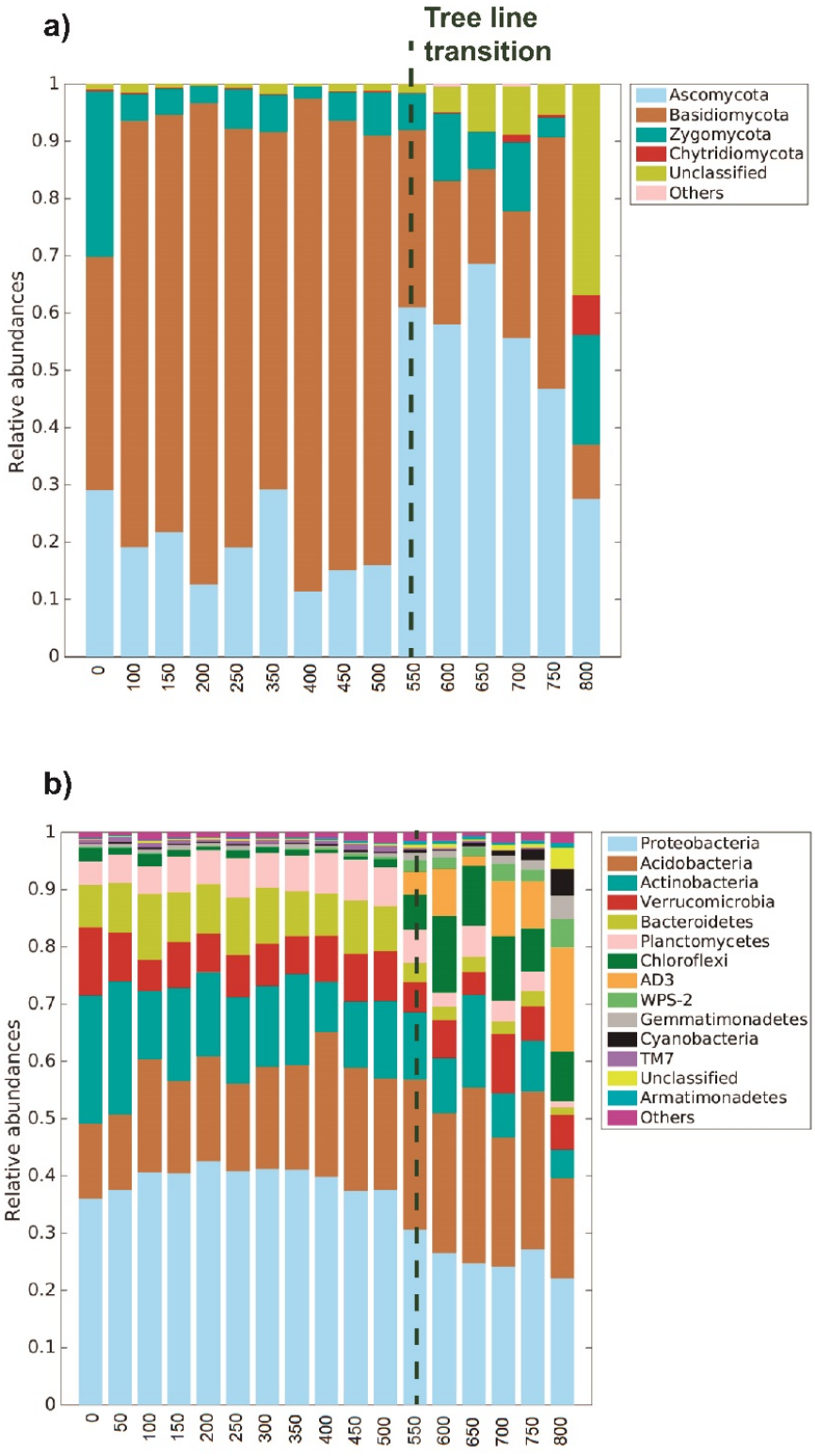


Figure S4: Relative abundance of the soil most abundant phyla (covering over 95% of total sequences) obtained from Illumina MiSeq sequencing of fungal ITS (a) and bacterial 16S (b) amplicons along the elevational gradient studied. The relative abundance fraction of other minor genera were summed, and are shown here as "Others". Dashed vertical lines represent tree line transition.

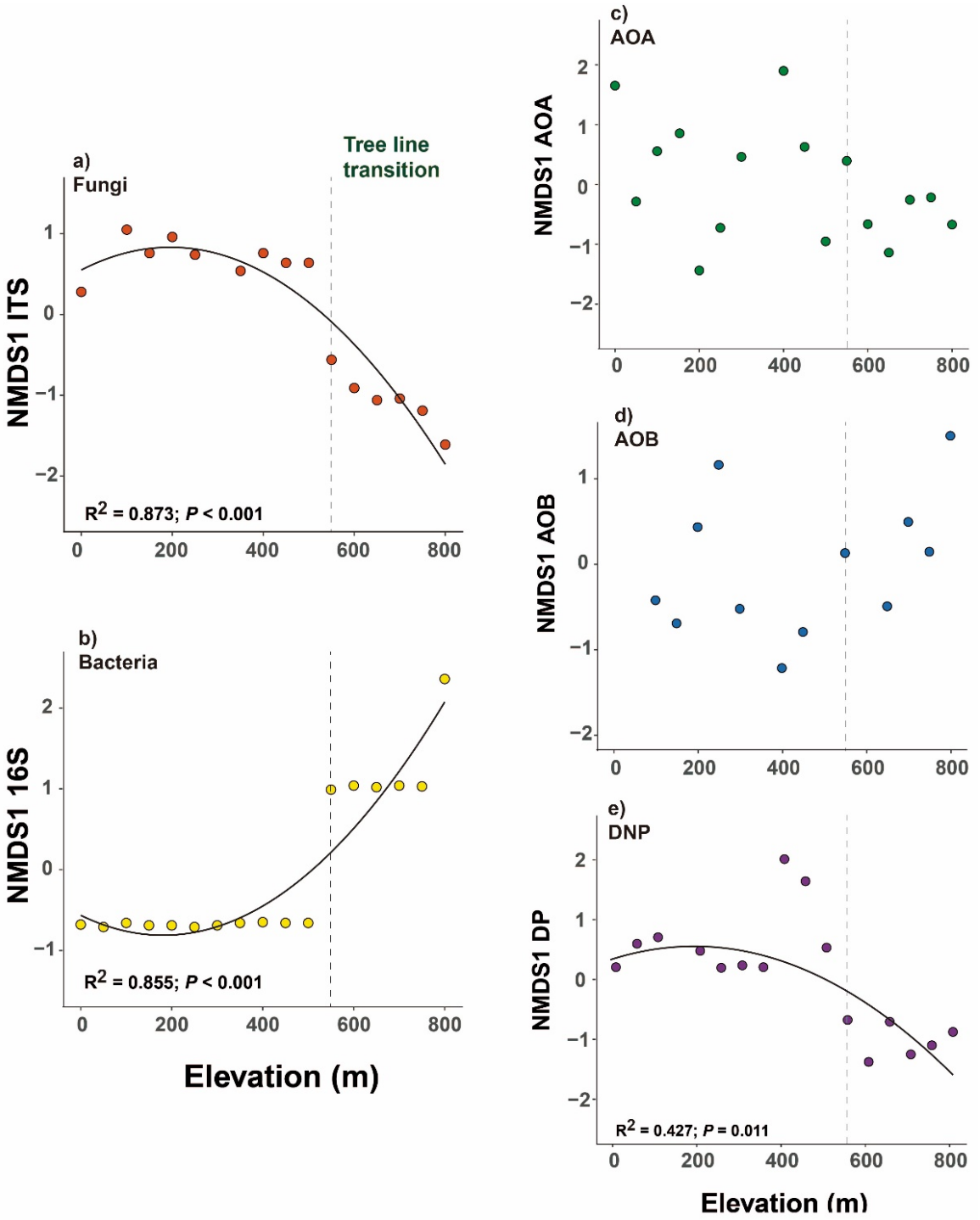


Figure S5: Regressions between elevation and the first axis obtained from a nMDS ordination for taxonomic and functional diversity. a) Fungi; b) Bacteria; c) ammonia oxidizing archaea (AOA); d) ammonia oxidizing bacteria (AOB); e) denitrifying prokaryotes (DNP).

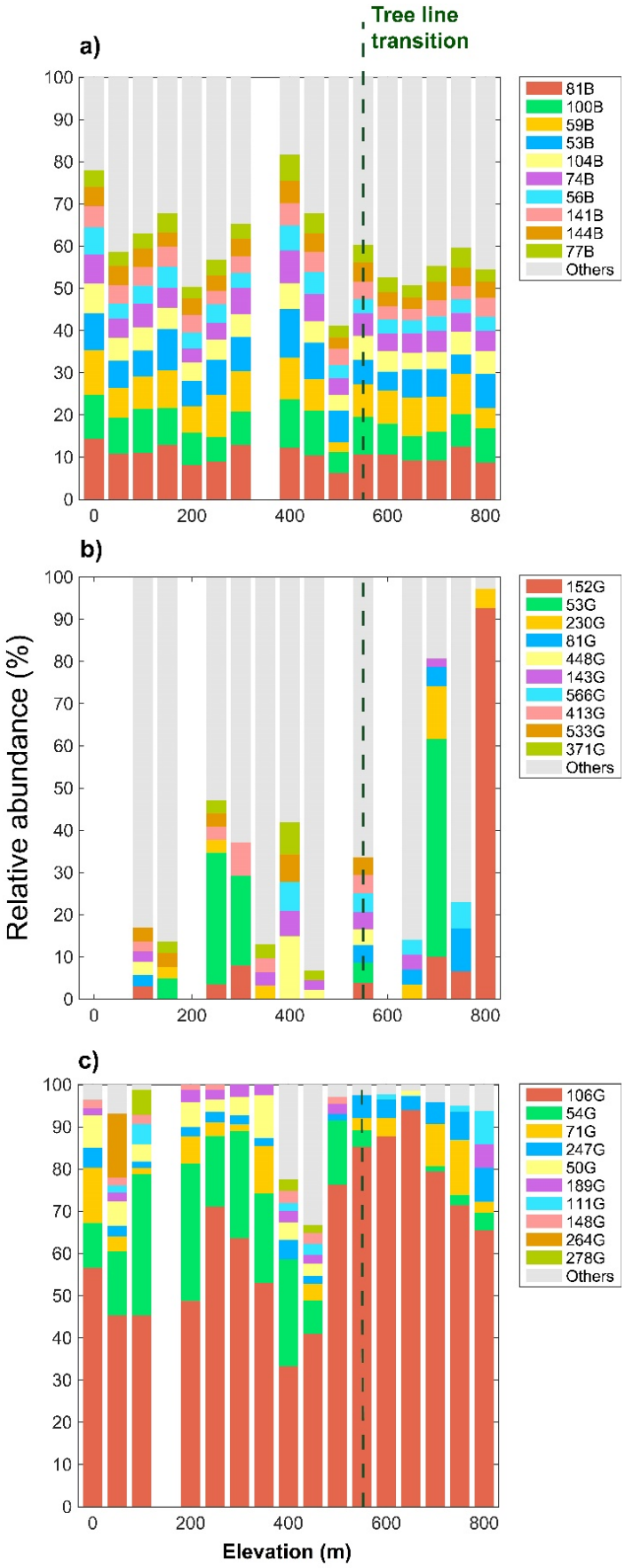


Figure S6: Relative abundance of the top 10 soil most abundant fragments (T-RFs) obtained from a T-RFLP analysis of archaeal (a) and bacterial (b) *amoA* and bacterial *nosZ* (c) amplicons, along the elevational gradient studied. The relative abundance fraction of other minor fragments were summed, and are shown here as "Others". Dashed vertical lines represent the tree line transition.

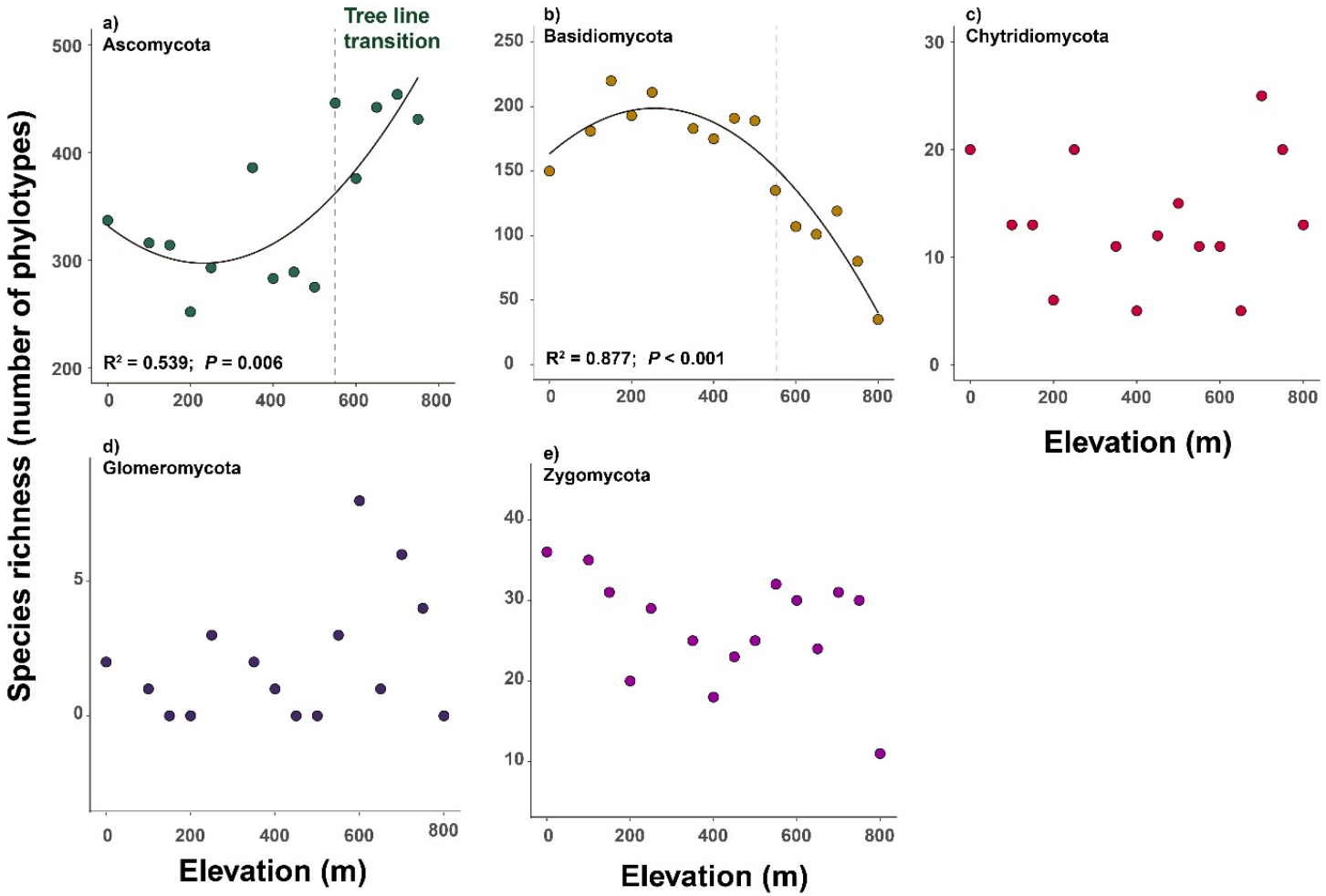


Figure S7: Taxonomic richness of the soil most abundant fungal phyla (number of phylotypes obtained from Illumina MiSeq sequencing of fungal ITS amplicons) along the elevational gradient studied. Model fit statistics and AICc values describing the relationship between elevation and richness of fungal phyla are shown in Table S5.

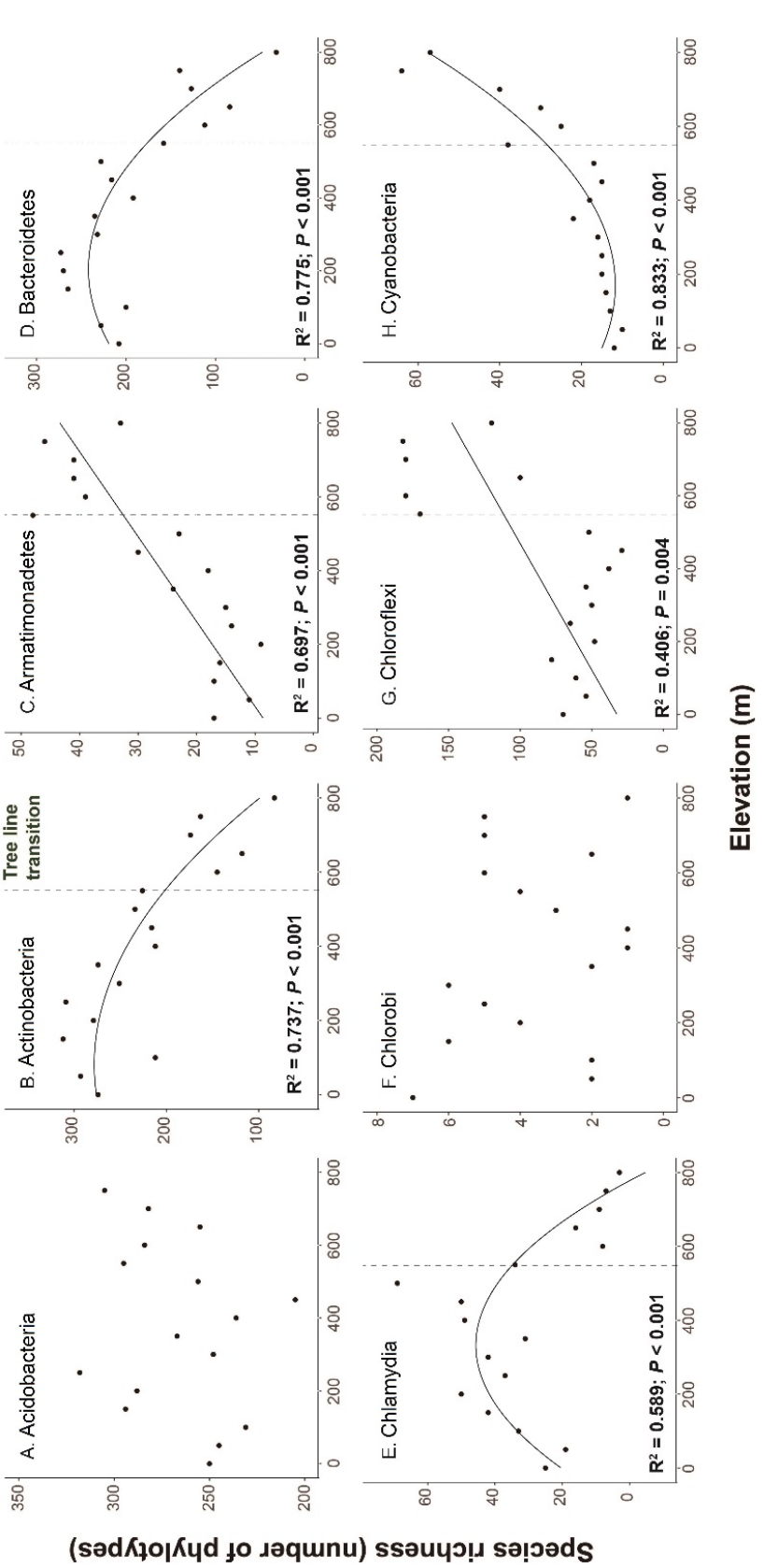


Figure S8: Taxonomic richness of the soil most abundant bacterial phyla (number of phylotypes obtained from Illumina MiSeq sequencing of bacterial 16S amplicons) along the elevational gradient studied. Model fit statistics and AICc values describing the relationship between elevation and richness of bacterial phyla are shown in Table S5.

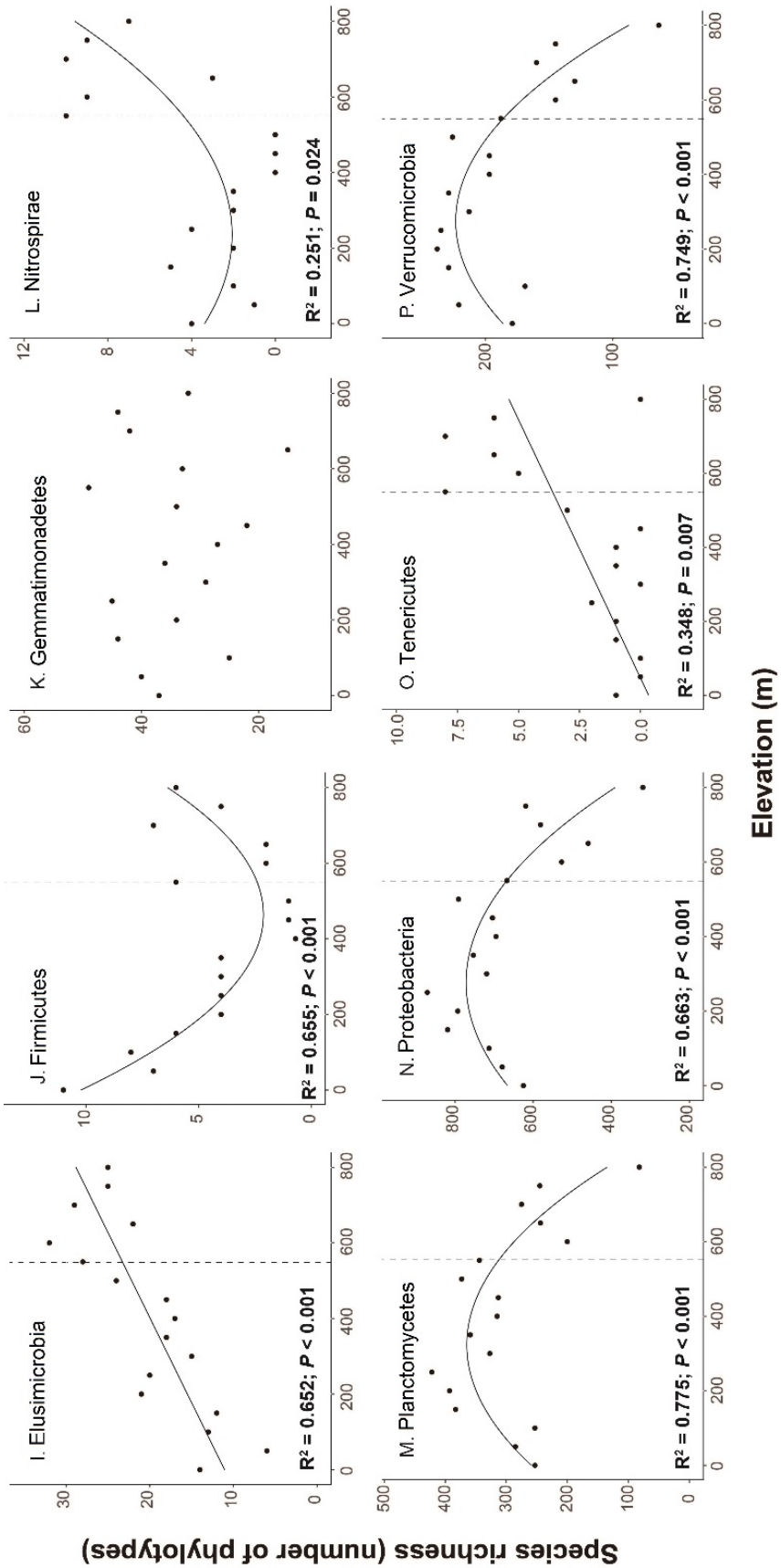


Figure S8: Continuation.

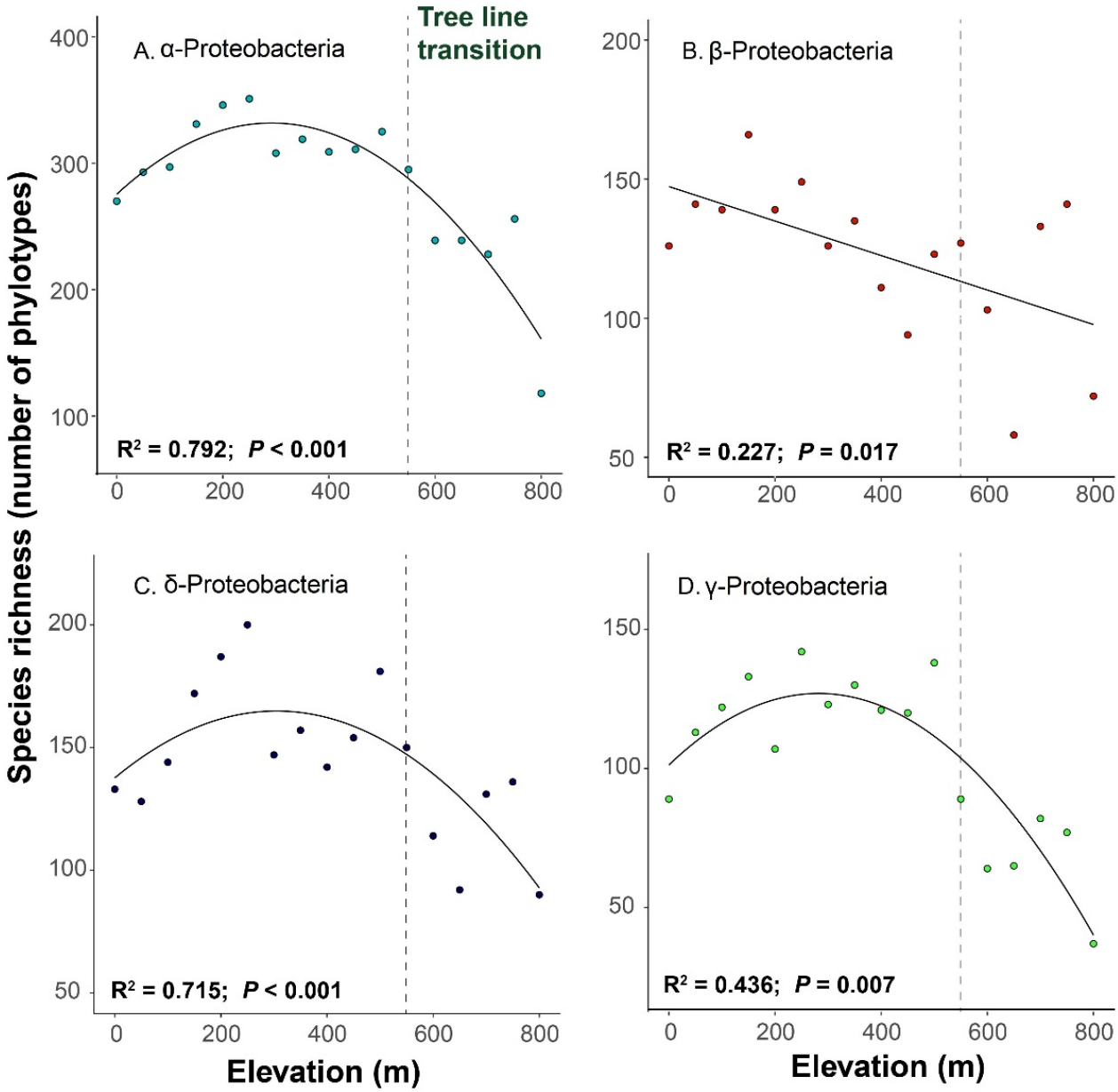


Figure S9: Taxonomic richness of soil *Proteobacteria* classes (number of phylotypes obtained from Illumina MiSeq sequencing of bacterial 16S amplicons) along the elevational gradient studied. Model fit statistics and AICc values describing the relationship between elevation and richness of Proteobacteria classes are shown in Table S5.

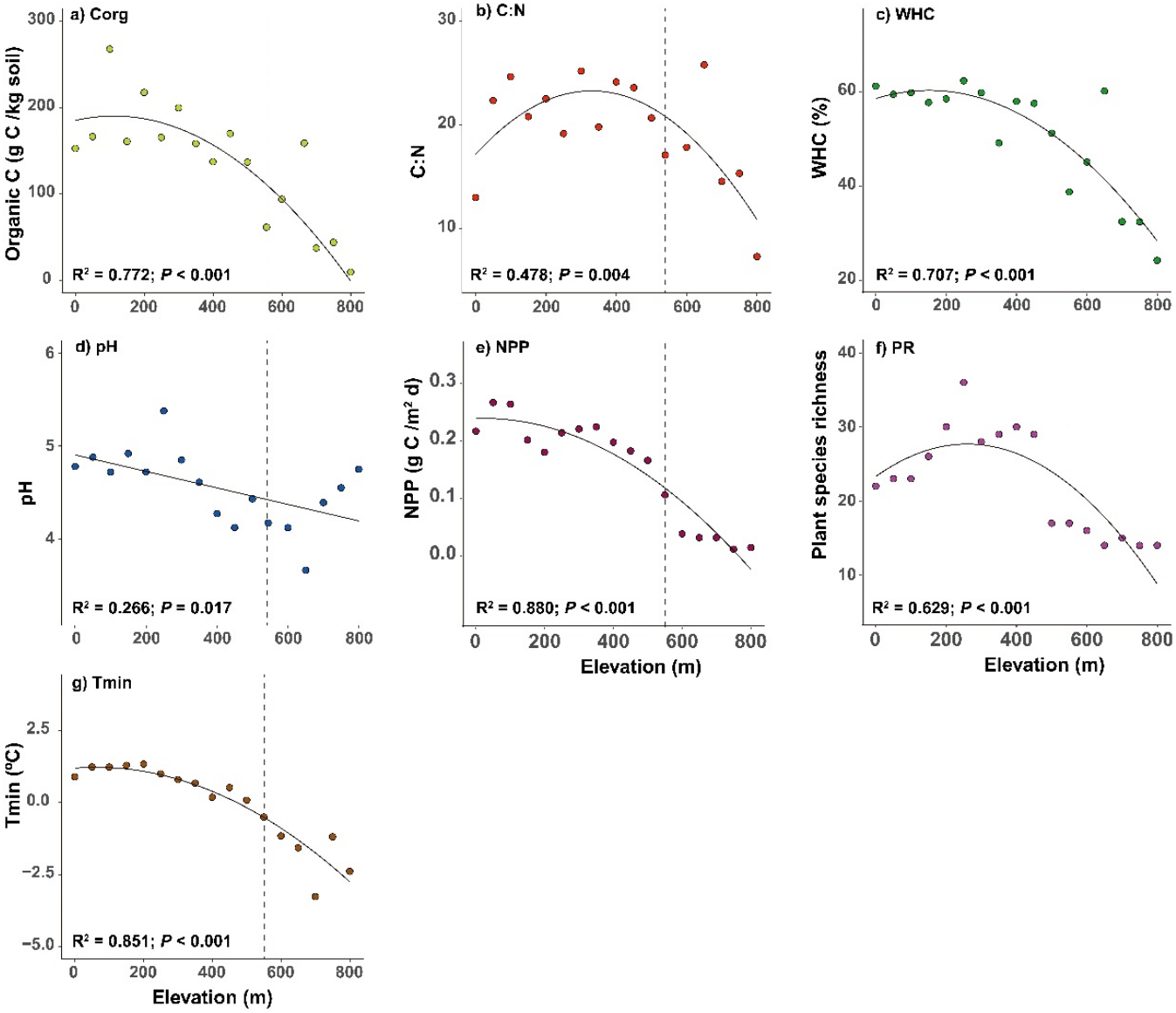


Figure S10: Regressions between elevation and the main predictors used in Random Forest analysis (excluding the categorical Habitat Change predictor). C: soil total carbon; C:N: soil Carbon:Nitrogen ratio; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness; Tmin: average of minimum daily temperatures. Model fit statistics and AICc values describing the relationship between elevation and predictors are shown in Table S6.

Table S1: Spearman rank correlations between measured climatic, soil and biotic variables. EC: electrical conductivity; WHC: soil water holding capacity; N: soil total N; C: soil total C; T: mean daily temperature; Tmax: mean daily maximum temperature; Tmin: mean daily minimum temperature; PR: plant richness. NPP: net primary productivity. * = *P*-valor < 0.05; ** = *P*-valor < 0.01. Bold characters represent correlation coefficients higher than 0.8 (collinearity).

	pH	**EC**	**WHC**	**N**	**C**	**T**	**Tmax**	**Tmin**	**C:N**	**PR**	**NPP**
Elevation	-0.615**	**-0.819****	-0.728**	-0.637**	-0.610**	**-0.975****	-0.748**	**-0.926****	-0.326	-0.648**	**-0.887****
pH		0.498*	0.378	0.207	0.128	0.550*	0.469	0.647**	-0.118	0.364	0.557*
EC			0.734**	0.593*	0.679**	0.784**	0.571*	**0.860****	0.701**	0.716**	**0.800****
WHC				**0.866****	0.759**	0.678**	0.655**	0.623**	0.552*	0.496*	0.615**
N					**0.951****	0.641**	0.694**	0.520*	0.537*	0.413	0.593*
C						0.620**	0.610**	0.542*	0.711**	0.425	0.569*
T							0.796**	**0.907****	0.325	0.646**	**0.893****
Tmax								0.625**	0.238	0.462	0.779**
Tmin									0.385	0.736**	0.789**
C:N										0.420	0.390
PR											0.675**

Table S2: Statistics and AICc values of the model fit of the relationships between the different microbial attributes (richness and abundance) and elevation and the main predictors obtained by Random Forest analysis (excluding the categorical Habitat Change predictor). C: soil total carbon; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness; Tmin: average of minimum daily temperatures; AOA: ammonia oxidizing archaea; AOB: ammonia oxidizing bacteria; DP: denitrifying prokaryotes.

Group		Predictor	Model	Adj-R^2	P	AICc	ΔAICc	Selected Model
Fungi	Richness	Elevation	Linear	0.351	0.015	164.76	5.82	
			Quadratic	0.595	0.003	158.94	**0.00**	✓
		C	Linear	0.081	0.1682	169.62	15.33	
			Quadratic	0.709	<0.001	154.29	**0.00**	✓
		WHC	Linear	0.569	0.001	159.00	2.36	
			Quadratic	0.656	0.001	156.64	**0.00**	✓
		NPP	Linear	0.469	0.004	161.94	1.89	✓
			Quadratic	0.561	0.004	160.05	**0.00**	
		PR	Linear	0.424	0.007	163.09	0.91	✓
			Quadratic	0.489	0.010	162.18	**0.00**	
		Tmin	Linear	0.593	<0.001	158.22	**0.00**	✓
			Quadratic	0.578	0.003	159.50	1.28	
	Abundance	Elevation	Linear	0.197	0.042	51.60	16.50	
			Quadratic	0.710	<0.001	35.10	**0.00**	✓
		C	Linear	0.362	0.006	47.73	9.33	
			Quadratic	0.649	<0.001	38.40	**0.00**	✓
		C:N	Linear	0.426	0.003	45.86	**0.00**	✓
			Quadratic	0.416	0.009	46.99	1.13	
		WHC	Linear	0.580	<0.001	40.65	1.4	✓
			Quadratic	0.631	<0.001	39.25	**0.00**	
		NPP	Linear	0.276	0.018	49.83	10.99	
			Quadratic	0.638	<0.001	38.84	**0.00**	✓
		PR	Linear	0.469	0.001	44.59	**0.00**	✓
			Quadratic	0.453	0.006	45.91	1.32	
Bacteria	Richness	Elevation	Linear	0.211	0.036	251.86	6.95	
			Quadratic	0.501	0.003	244.91	**0.00**	✓
		NPP	Linear	0.279	0.017	250.33	4.74	
			Quadratic	0.480	0.004	245.59	**0.00**	✓
		PR	Linear	0.330	0.009	249.07	**0.00**	✓
			Quadratic	0.289	0.036	250.92	1.85	
		Tmin	Linear	0.364	0.006	248.18	**0.00**	✓
			Quadratic	0.326	0.025	250.00	1.82	
	Abundance	Elevation	Linear	0.111	0.104	39.32	3.82	
			Quadratic	0.317	0.027	35.50	**0.00**	✓
		C:N	Linear	0.339	0.008	34.13	**0.00**	✓
			Quadratic	0.324	0.024	35.19	1.06	
		PR	Linear	0.310	0.012	34.85	**0.00**	✓
			Quadratic	0.265	0.045	36.77	1.92	
AOA	Richness	Elevation	Linear	0,082	0,148	111,76	**0,00**	✓
			Quadratic	0,012	0,366	113,75	2,00	
		NPP	Linear	0,158	0,070	110,37	**0,00**	✓

			Quadratic	0,105	0,192	112,17	1,80	
	Abundance	Elevation	Linear	0,071	0,988	5,33	**0,00**	✓
			Quadratic	0,147	0,963	7,23	1,90	
AOB	Richness	Elevation	Linear	0,196	0,084	94,38	**0,00**	✓
			Quadratic	0,134	0,213	96,01	1,63	
	Abundance	Elevation	Linear	0,455	0,002	17,47	4,76	
			Quadratic	0,606	<0,001	12,71	**0,00**	✓
		C	Linear	0,393	0,004	19,35	1,10	✓
			Quadratic	0,457	0,005	18,25	**0,00**	
		WHC	Linear	0,527	<0,001	15,06	**0,00**	✓
			Quadratic	0,499	0,003	16,90	1,84	
		NPP	Linear	0,611	<0,001	11,82	1,70	✓
			Quadratic	0,665	<0,001	10,13	**0,00**	
		PR	Linear	0,443	0,002	17,77	0,51	✓
			Quadratic	0,485	0,004	17,26	**0,00**	
		Tmin	Linear	0,513	<0,001	15,65	**0,00**	✓
			Quadratic	0,504	0,003	16,80	1,15	
DP	Richness	Elevation	Linear	0,122	0,120	62,90	5,55	
			Quadratic	0,441	0,016	57,35	**0,00**	✓
		NPP	Linear	0,094	0,151	63,33	5,58	
			Quadratic	0,425	0,019	57,75	**0,00**	✓
		Tmin	Linear	0,038	0,243	64,18	**0,00**	✓
			Quadratic	0,063	0,278	64,58	0,40	
	Abundance	Elevation	Linear	0,245	0,029	31,36	2,33	
			Quadratic	0,297	0,039	29,03	**0,00**	✓

Table S3: Statistics and AICc values of the model fit of the relationships between the different microbial ecological and functional clusters and elevation and the main predictors obtained by Random Forest analysis (excluding the categorical Habitat Change predictor). C: soil total carbon; C:N: soil Carbon:Nitrogen ratio; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness; Tmin: average of minimum daily temperatures.

Group	Predictor	Model	Adj-R^2	P	AICc	ΔAICc	Selected Model
Taxonomic Cluster#0	Elevation	Linear	0.685	<0.001	13.41	4.13	
		Quadratic	0.773	<0.001	9.28	**0.00**	✓
	C	Linear	0.141	0.111	28.47	12.74	
		Quadratic	0.652	<0.001	15.73	**0.00**	✓
	C:N	Linear	0.099	0.148	29.17	**0.00**	✓
		Quadratic	0.114	0.152	29.72	0.55	
	WHC	Linear	0.474	0.005	21.12	**0.00**	✓
		Quadratic	0.432	0.016	23.05	1.93	
	NPP	Linear	0.834	<0.001	3.80	**0.00**	✓
		Quadratic	0.841	<0.001	3.95	0.15	
	PR	Linear	0.676	<0.001	13.83	2.46	
		Quadratic	0.739	<0.001	11.37	**0.00**	✓
	Tmin	Linear	0.780	<0.001	4.86	**0.00**	✓
		Quadratic	0.831	<0.001	8.06	3.20	
Taxonomic Cluster#1	Elevation	Linear	0.610	0.001	14.13	3.84	
		Quadratic	0.714	<0.001	10.29	**0.00**	✓
	C	Linear	0.256	0.031	23.83	8.80	
		Quadratic	0.608	0.001	15.03	**0.00**	✓
	WHC	Linear	0.550	0.002	16.30	**0.00**	✓
		Quadratic	0.513	0.003	18.27	1.97	
	NPP	Linear	0.859	<0.001	-1.11	**0.00**	✓
		Quadratic	0.863	<0.001	-0.73	0.38	
	PR	Linear	0.649	0.002	12.53	4.34	
		Quadratic	0.751	<0.001	8.19	**0.00**	✓
	Tmin	Linear	0.786	<0.001	5.13	**0.00**	✓
		Quadratic	0.797	<0.001	5.18	0.05	
Taxonomic Cluster#2	Elevation	Linear	0.055	0.667	23.85	2.96	
		Quadratic	0.179	0.121	20.89	**0.00**	✓
	C:N	Linear	0.503	0.001	12.57	**0.00**	✓
		Quadratic	0.468	0.006	14.38	1.81	
	pH	Linear	0.236	0.038	19.01	**0.00**	✓
		Quadratic	0.174	0.163	20.98	1.97	
	Tmin	Linear	0.036	0.275	22.50	1.13	✓
		Quadratic	0.152	0.115	21.37	**0.00**	
Functional Cluster#0	Elevation	Linear	0.048	0.541	9.50	**0.00**	✓
		Quadratic	0.117	0.849	11.26	1.76	
Functional Cluster#1	Elevation	Linear	0.380	0.008	3.89	**0.00**	✓

		Quadratic	0.345	0.029	5.52	1.63	
Functional Cluster#2	Elevation	Linear	0.124	0.082	15.46	**0.00**	✓
		Quadratic	0.094	0.203	16.77	1.31	
Functional Cluster#3	Elevation	Linear	0.062	0.706	38.13	**0.00**	✓
		Quadratic	0.124	0.925	39.91	1.78	
Functional Cluster#4	Elevation	Linear	0.050	0.615	11.79	**0.00**	✓
		Quadratic	0.117	0.815	13.58	1.79	

Table S4: Statistics and AICc values of the model fit to the relationships between the different fungal functional groups considered and the main predictors obtained from Random Forest analysis (excluding the categorical Habitat Change predictor). C: soil total carbon; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness; Tmin: average of minimum daily temperatures.

Group	Predictor	Model	Adj-R^2	P	AICc	ΔAICc	Selected Model
Animal pathogen	Elevation	Linear	0.451	<0.001	-27.49	3.99	
		Quadratic	0.601	0.008	-31.48	**0.00**	✓
	WHC	Linear	0.139	0.040	-20.76	0.12	✓
		Quadratic	0.191	0.102	-20.88	**0.00**	
	NPP	Linear	0.176	0.089	-21.41	**0.00**	✓
		Quadratic	0.122	0.126	-19.65	1.76	
	pH	Linear	0.292	0.023	-23.69	**0.00**	✓
		Quadratic	0.282	0.188	-22.67	1.02	
	Tmin	Linear	0.148	0.072	-20.90	**0.00**	✓
		Quadratic	0.150	0.113	-20.14	0.76	
Arbuscular Mycorrhizal	Elevation	Linear	0.056	0.686	-10.02	**0.00**	✓
		Quadratic	0.020	0.404	-8.05	1.97	
Ectomycorrhizal	Elevation	Linear	0.350	0.012	130.97	5.01	
		Quadratic	0.559	0.003	125.96	**0.00**	✓
	C:N	Linear	0.318	0.017	131.70	**0.00**	✓
		Quadratic	0.261	0.065	133.69	1.99	
	NPP	Linear	0.705	<0.001	119.11	**0.00**	✓
		Quadratic	0.697	<0.001	120.31	1.20	
	PR	Linear	0.514	0.002	126.60	4.13	
		Quadratic	0.650	<0.001	122.47	**0.00**	✓
	Tmin	Linear	0.578	<0.001	124.49	**0.00**	✓
		Quadratic	0.561	0.003	125.88	1.38	
Endophyte	Elevation	Linear	0.230	0.062	77.89	**0.00**	✓
		Quadratic	0.167	0.116	79.87	1.98	
	C	Linear	0.017	0.748	82.07	0.14	✓
		Quadratic	0.045	0.297	81.93	**0.00**	
	WHC	Linear	0.074	0.140	80.67	0.76	✓
		Quadratic	0.165	0.138	79.91	**0.00**	
	NPP	Linear	0.396	0.006	74.26	**0.00**	✓
		Quadratic	0.380	0.023	75.46	1.19	
	PR	Linear	0.347	0.018	75.42	**0.00**	✓
		Quadratic	0.330	0.038	76.60	1.18	
	Tmin	Linear	0.236	0.035	77.78	2.46	
		Quadratic	0.385	0.027	75.33	**0.00**	✓
Ericoid Mycorrhizal	Elevation	Linear	0.251	0.027	58.12	0.23	✓
		Quadratic	0.301	0.044	57.89	**0.00**	
	pH	Linear	0.273	0.033	57.67	**0.00**	✓
		Quadratic	0.230	0.061	59.34	1.67	

	Tmin	Linear	0.345	0.016	56.10	0.73	✓
		Quadratic	0.409	0.016	55.37	**0.00**	
Fungal Parasite	Elevation	Linear	0.242	0.053	-31.30	**0.00**	✓
		Quadratic	0.180	0.113	-29.33	1.97	
	pH	Linear	0.006	0.266	-27.23	2.09	
		Quadratic	0.180	0.105	-29.32	**0.00**	✓
	Tmin	Linear	0.162	0.063	-29.80	**0.00**	✓
		Quadratic	0.162	0.106	-28.99	0.81	
Lichenized	Elevation	Linear	0.246	0.029	47.44	1.03	✓
		Quadratic	0.333	0.059	46.41	**0.00**	
	NPP	Linear	0.318	<0.001	45.93	**0.00**	✓
		Quadratic	0.319	0.069	46.71	0.79	
	PR	Linear	0.208	0.027	48.17	1.17	✓
		Quadratic	0.306	0.073	47.00	**0.00**	
	Tmin	Linear	0.310	0.025	46.11	**0.00**	✓
		Quadratic	0.257	0.071	48.02	1.90	
Plant Pathogen	Elevation	Linear	0.188	0.052	64.08	0.38	✓
		Quadratic	0.250	0.112	63.70	**0.00**	
Soil Saprotroph	Elevation	Linear	0.067	0.725	18.80	2.28	
		Quadratic	0.131	0.174	16.52	**0.00**	✓
Wood Saprotroph	Elevation	Linear	0.027	0.373	-17.59	**0.00**	✓
		Quadratic	0.021	0.634	-16.88	0.71	

Table S5: Model fit statistics and AICc values for elevation and the richness of major bacterial and fungal groups obtained from Illumina MiSeq sequencing of bacterial 16S and fungal ITS amplicons.

Group	Model	Adj-R^2	P	AICc	ΔAICc	Selected Model
Acidobacteria	Linear	0.062	0.798	175.85	0.00	✓
	Quadratic	0.080	0.674	176.97	1.12	
Actinobacteria	Linear	0.668	<0.001	176.47	3.11	
	Quadratic	0.737	<0.001	173.36	0.00	✓
Armatimonadetes	Linear	0.697	<0.001	118.89	0.00	✓
	Quadratic	0.686	<0.001	120.35	1.46	
Bacteroidetes	Linear	0.587	<0.001	181.10	9.53	
	Quadratic	0.775	<0.001	171.57	0.00	✓
Chlamydiia	Linear	0.125	0.090	149.31	12.02	
	Quadratic	0.589	<0.001	137.29	0.00	✓
Chlorobi	Linear	0.015	0.397	75.43	0.00	✓
	Quadratic	0.060	0.592	77.00	1.57	
Chloroflexi	Linear	0.406	0.004	179.34	1.70	✓
	Quadratic	0.488	0.004	177.64	0.00	
Cyanobacteria	Linear	0.678	<0.001	127.24	10.34	
	Quadratic	0.833	<0.001	116.89	0.00	✓
Elusimicrobia	Linear	0.652	<0.001	99.53	0.00	✓
	Quadratic	0.643	<0.001	100.77	1.24	
Firmicutes	Linear	0.123	0.092	85.79	15.02	
	Quadratic	0.655	<0.001	70.77	0.00	✓
Gematimonadetes	Linear	0.062	0.791	128.12	0.00	✓
	Quadratic	0.105	0.791	129.64	1.52	
Nitrospirae	Linear	0.251	0.024	90.63	1.52	✓
	Quadratic	0.347	0.020	89.11	0.00	
Planctomicetes	Linear	0.170	0.056	199.34	12.54	
	Quadratic	0.622	<0.001	186.80	0.00	✓
Proteobacteria	Linear	0.357	0.007	212.20	10.13	
	Quadratic	0.663	<0.001	202.07	0.00	✓
Spirohaetes	Linear	0.047	0.200	84.41	0.00	✓
	Quadratic	-0.014	0.433	86.30	1.89	
Tenericutes	Linear	0.348	0.007	80.99	0.00	✓
	Quadratic	0.302	0.032	82.99	2.00	
Verrucomicrobia	Linear	0.408	0.003	173.95	13.74	
	Quadratic	0.749	<0.001	160.21	0.00	✓
α_Proteobacteria	Linear	0.366	0.006	181.66	18.09	
	Quadratic	0.792	<0.001	163.57	0.00	✓
β_Proteobacteria	Linear	0.277	0.017	159.38	0.00	✓
	Quadratic	0.229	0.064	161.31	1.93	
γ_Proteobacteria	Linear	0.366	0.006	160.36	12.77	
	Quadratic	0.715	<0.001	147.59	0.00	✓
δ_Proteobacteria	Linear	0.168	0.058	164.85	5.79	
	Quadratic	0.436	0.007	159.06	0.00	✓
Ascomycota	Linear	0.372	0.012	156.59	3.56	
	Quadratic	0.539	0.006	153.03	0.00	✓

Basidiomycota	Linear	0.562	<0.001	153.37	18.27	
	Quadratic	0.877	<0.001	135.11	**0.00**	✓
Chytridiomycota	Linear	0.074	0.863	100.62	1.35	✓
	Quadratic	0.069	0.258	99.27	**0.00**	
Glomeromycota	Linear	0.053	0.205	71.93	**0.00**	✓
	Quadratic	0.024	0.458	73.90	1.97	
Zygomycota	Linear	0.100	0.134	102.21	**0.00**	✓
	Quadratic	0.056	0.281	103.73	1.52	

Table S6: Statistics and AICc values of the model fit of the relationships between elevation and the main predictors used in Random Forest analysis (excluding the categorical Habitat Change predictor). C: soil total carbon; C:N: soil Carbon:Nitrogen ratio; WHC: soil water holding capacity; NPP: net primary productivity; PR: plant richness; Tmin: average of minimum daily temperatures.

Variable	Model	Adj-R^2	P	AICc	ΔAICc	Selected Model
Corg	Linear	0.674	<0.001	167.36	4.92	
	Quadratic	0.772	<0.001	162.44	**0.00**	✓
C:N	Linear	0.103	0.065	104.93	8.38	
	Quadratic	0.478	0.004	96.55	**0.00**	✓
WHC	Linear	0.599	<0.001	121.28	4.53	
	Quadratic	0.707	<0.001	116.75	**0.00**	✓
pH	Linear	0.266	0.017	16.00	**0.00**	✓
	Quadratic	0.271	0.041	16.70	0.70	
NPP	Linear	0.815	<0.001	-58.08	6.57	
	Quadratic	0.880	<0.001	-64.65	**0.00**	✓
PR	Linear	0.382	0.004	110.41	7.85	
	Quadratic	0.629	<0.001	102.56	**0.00**	✓
Tmin	Linear	0.773	<0.001	38.41	6.27	
	Quadratic	0.851	<0.001	32.14	**0.00**	✓

CAPÍTULO 4: Identity of plant, lichen and moss species connects with microbial abundance and soil functioning in Maritime Antarctica

Abstract

Background and aims

We lack studies evaluating how the identity of plant, lichen and moss species relates to microbial abundance and soil functioning on Antarctica. If species identity is associated with soil functioning, distributional changes of key species, linked to climate change, could significantly affect Antarctic soil functioning.

Methods

We evaluated how the identity of six Antarctic plant, lichen and moss species relates to a range of soil attributes (C, N and P cycling), microbial abundance and structure in Livingston Island, Maritime Antarctica. We used an effect size metric to predict the association between species (vs. bare soil) and the measured soil attributes.

Results

We observed species-specific effects of the plant and biocrust species on soil attributes and microbial abundance. Phenols, phosphatase and β-D-cellobiosidase activities were the most important attributes characterizing the observed patterns. We found that the evaluated species positively correlated with soil nutrient availability and microbial abundance vs. bare soil.

Conclusions

We provide evidence, from a comparative study, that plant and biocrust identity is associated with different levels of soil functioning and microbial abundance in Maritime Antarctica. Our results suggest that changes in the spatial distribution of these species linked to climate change could potentially entail changes in the functioning of Antarctic terrestrial ecosystems.

Keywords: Antarctic vegetation, bacteria, fungi, qPCR, soil enzyme activities.

Introduction

Current exposed lands in Antarctica, mainly located at coastal regions or rock ridges, are the habitat for Antarctic plants and cryptogamic species –including the Antarctic flowering plants *Deschampsia antarctica* and *Colobanthus quitensis*, and multiple bryophyte and lichen species, some of them forming biocrust communities. In these areas, Antarctic vegetation shows a spatial patchy distribution as a consequence of multiple ecological conditions (Kappen *et al.*, 1985; Melick & Seppelt, 1997). The establishment and distribution of Antarctic vegetation is primarily conditioned by ice and snow melt at some point during the year, and thus is ultimately determined by microclimatic factors (Kennedy, 1993; Hughes *et al.*, 2006; Vieira *et al.*, 2014). These factors (e.g. moisture or texture micro-gradients) also affect soil properties and may compromise successful propagule colonization (Bergstrom *et al.*, 2006), but the irruption of vegetation can have direct consequences on soil functioning. For instance, we know that the presence of cryptogams is positively associated with fertility islands (*sensu* Schlesinger et al. 1996) leading to higher N and C concentration underneath them in continental Antarctic soils (Cannone et al. 2008) and elsewhere (Delgado-Baquerizo *et al.*, 2016a). However, the species-specific association of plant, lichen and moss species identity with multiple soil attributes, nutrient cycling and microbial abundance (Cornelissen *et al.*, 2007; Mallen-Cooper & Eldridge, 2016) remains largely unexplored in Antarctica.

The role of plant, lichen and moss species identity (at both functional and taxonomic levels) as a potential environmental predictor of soil functioning is well-known in terrestrial ecosystems (Hooper & Vitousek, 1997; Chen & Stark, 2000; Chapman *et al.*, 2005; Fan *et al.*, 2011). Primary productivity, nitrogen (N) fixation capacity or thallus/stem structure are known to be involved in litter quality and quantity production or dust capture, important factors involved in the formation of the so called fertility islands in patchy vegetated ecosystems (e.g. arid ecosystems; de Graaff et al. 2014; Ochoa-Hueso et al. 2018). Similarly, recent studies have stated that the identity of biocrusts has important implications for both microbial communities and soil functioning in drylands (Concostrina-Zubiri *et al.*, 2013; Delgado-Baquerizo *et al.*, 2015; Liu *et al.*, 2016, 2017). Therefore, not only the presence but also the identity of plant and biocrust (i.e. lichens and bryophytes) species may differentially influence both microbial communities and soil functioning. However, studies providing evidence for a link between species identity and soil attributes are largely lacking, limiting our capacity to predict how ongoing changes in the coverage, relative abundance, and metabolic activity of these plant and biocrust species with climate change (e.g. Torres-Mellado et al.

2011; Cannone et al. 2016; Amesbury et al. 2017) may potentially impact the functioning of Antarctic terrestrial ecosystems.

Using a comparative approach, we evaluated how four lichen species (*Leptogium puberulum, Stereocaulon alpinum, Sphaerophorus globosus*, and *Cladonia* sp.), one moss (*Sanionia uncinata*), and the most common Antarctic flowering-plant (*Deschampsia antarctica*) (Sancho *et al.*, 1999; Søchting *et al.*, 2004) are associated with multiple soil attributes related to carbon, nitrogen and phosphorus cycling and the abundance of soil fungi and bacteria at Livingston Island (Antarctic Peninsula). The expected changes in the relative abundance of these species due to, for example climate change, could alter soil functioning in this continent (Kardol *et al.*, 2010; van der Putten *et al.*, 2016; Liu *et al.*, 2017). Alternatively, if vegetation patterns do not relate to soil functioning, changes in their relative abundance in response to changing climatic conditions may not entail differentiated changes in soil functioning in the region. We hypothesized that the identity of plant and biocrust species will largely relate to different levels of multiple soil attributes (i.e. concentration and cycling of soil nutrients) and the abundance of soil fungi and bacteria. To test this, we first evaluated whether soil functioning and microbial abundance differed under monospecific patches of the plant and biocrust species evaluated. We then characterized which variations associated with the observed differences. Additionally, we determined which soil attributes were more sensitive to species identity. Finally, we evaluated the differences in soil functioning between the different species evaluated and areas devoid of vegetation. Our study is among the first exploring the species-specific connections of Antarctic vegetation (i.e. lichens, bryophyte and vascular species) with below-ground soil functioning. Advancing our knowledge on the relationships between the identity of vegetation components and soil biochemistry and microbial communities in Antarctica is critical to understand this ecosystem and to accurately predict potential impacts of changes in the relative abundance of these micro-habitats because of global change.

Materials and Methods

Site description

The study was carried out in the vicinity of the Juan Carlos I Spanish Antarctic Base (62°39′46″S 60°23′20″W), which is located on Livingston Island, 120 km north of the Antarctic Peninsula. Geology is primarily composed of acidic sedimentary, metamorphic, plutonic and volcanic rocks. The climate of Livingston Island is cold maritime, with mean

summer temperature around 1 ºC and minimum absolute temperature in winter not lower than -20°C (Sancho *et al.*, 2017). Annual precipitation is circa 445 mm, mainly concentrated in summer and autumn seasons (Bañón *et al.*, 2013). Around 10% of the surface of Livingston Island is free of ice during the summer season –mainly coastal strips and rocky ridges– allowing the development of plant and cryptogamic species. Vegetation in these areas is dominated by terricolous or saxicolous lichens (species of the genera *Buellia*, *Caloplaca*, *Cladonia*, *Leptogium*, *Pertusaria*, *Placopsis*, *Rhizocarpon, Stereocaulon* and *Usnea*, among others), and bryophytes (species of the genera *Andreaea*, *Brachythecium*, *Bryum*, *Polytrichum* and *Sanionia*, among others), in combination with the native flowering plants *Deschampsia antarctica* and *Colobanthus quitensis* (Sancho *et al.*, 1999; Søchting *et al.*, 2004).

Sampling design

Soil sampling was conducted at the end of the Antarctic summer of 2015. We selected six of the most common species of vegetation (Fig 1) growing on Livingston Island (Sancho *et al.*, 1999; Søchting *et al.*, 2004), including a flowering plant (*Deschampsia antarctica*,), four lichens (*Stereocaulon alpinum, Sphaerophorus globosus, Leptogium puberulum,* and *Cladonia* sp.), and one moss (*Sanionia uncinata*). We acknowledge that some selected species (i.e. *S. alpinum* and *S. globosus*) are not biocrusts *per se* but they grow forming dense and complex cryptogamic covers in combination with other biocrust forming species (e.g. *Ceratodon purpureus*, *Cladonia chlorophaea*, *Psoroma hypnorum*, *Placopsis contortuplicata*, *Ochrolechia frigida*). For simplicity, we use the term biocrust (*sensu lato*) throughout the manuscript to refer to these communities. *Leptogium puberulum* and *S. alpinum* are nitrogen fixing lichens including cyanobacteria (genus *Nostoc*) as a principal photobiont (*L. puberulum*) or as a secondary symbiont in cephalodia (*S. alpinum*). *Sphaerophorus globosus* and *Cladonia* sp. lack N-fixation capacity. The moss *S. uncinata* presents epiphytic cyanobacteria with lower fixation rates than that observed in abovementioned lichens (data not shown). *Deschampsia antarctica* was also included in our study because it has a significant presence in the studied area and coexists with the abovementioned biocrust species. From a total sampling area of 0.9 ha, we randomly selected single-species patches (having at least 10 cm diameter) for our soil sampling. A distance of at least 2 m was kept between patches to ensure spatial independency between samples (Delgado-Baquerizo *et al.*, 2013). For each species, ten replicated soil samples from the top 5 cm mineral soil profile were collected with a 5 x 5 cm core, with the

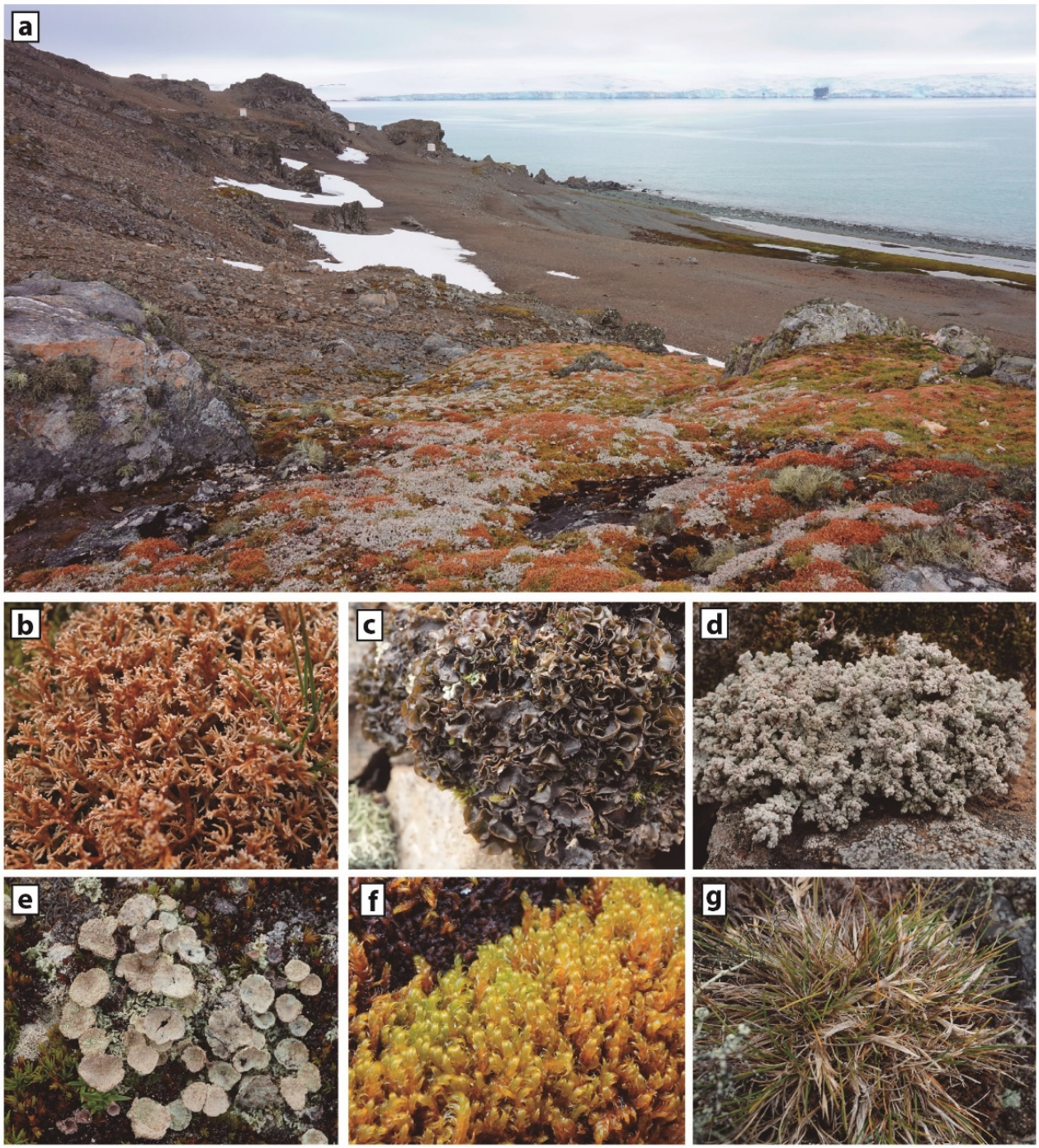


Figure 1: a): Raised beaches in the vicinity of Juan Carlos I Spanish Antarctic Base showing the vegetation communities sampled. Studied species: *Sphaerophorus globosus* (b), *Leptogium puberulum* (c), *Stereocaulon alpinum* (d), *Cladonia* sp. (e), *Sanionia uncinata* (f), and *Deschampsia antarctica* (g).

exception of *Cladonia* sp., which was more frequent on rocky substrates. This prevented us from obtaining more than six samples with soil profiles deeper than 5 cm for this species. Areas with no vegetation cover (bare soil) were used as controls (10 replicates). Thus, a total of 66 soil samples were collected, sieved with a 2 mm sieve and divided in two fractions. A fraction of soil was immediately frozen at -20 ºC for molecular analysis. The other fraction was air dried for biogeochemical analyses. Both fractions were transported to the laboratory of Rey Juan Carlos University in Móstoles (Spain) for analyses.

Measurement of C, N and P variables in soil

We measured in the laboratory a total of 16 soil variables linked to the stocks and cycling of C, N and P: dissolved organic C (Corg), phenols, α-Glucosidase (AG, starch degradation), β-Glucosidase (BG, starch degradation), β-D-Cellobiosidase (CB, cellulose degradation), total N, available nitrogen (AN), proteins, potential net mineralization and nitrification rates, microbial biomass N, Xylosidase (XYL, hemicellulose degradation), L-Leucine-aminopeptidase (LAP, protein degradation), N-acetyl-β-glucosaminidase (NAG, chitin degradation), dissolved inorganic P (DIP), and Phosphatase (PHOS, P mineralization). All these variables, referred as soil attributes hereafter (both including soil functions and properties), are either measurements of specific ecosystem processes (e.g. N mineralization rate) or key properties (e.g. organic C, total N, inorganic P and soil enzymes), which together constitute a good proxy of nutrient cycling, biological productivity and the build-up of nutrient pools (Reiss *et al.*, 2009; Jax, 2010; Maestre *et al.*, 2012b,a).

The N of microbial biomass (MB-N) was determined using the fumigation-extraction method of Brookes et al. (1985). Soil pH was measured for all of the soil samples with a pH-meter in a 1:2.5 mass/volume soil and water suspension. Sand, clay, and silt contents were determined according to Kettler et al. (2001). Electrical conductivity was determined using a conductivity meter in the laboratory. Soil moisture content was measured by oven-drying the samples at 105 ºC for 24 h, and soil water holding capacity was measured by gravimetry. Soil N was measured with a CN analyzer (Leco CHN628 Series; Leco Corporation, St Joseph, MI, USA). Organic C was determined following Anderson and Ingram (1993). Total available N (sum of ammonium, nitrate, and dissolved organic N) was colorimetrically analyzed from K_2SO_4 0.5 M soil extracts using a 1 : 5 soil/extract ratio as described by Delgado-Baquerizo et al. (2011). Phosphate was determined by colorimetry from a 0.5 M $NaHCO_3$ extraction (Bray & Kurtz, 1945).

We measured the potential activity of seven hydrolytic soil enzymes involved in the degradation of common organic matter constituents: α-Glucosidase (starch degradation; AG), β-Glucosidase (starch degradation; BG), β-D-Cellobiosidase (cellulose degradation; CB), L-Leucine aminopeptidase (protein degradation; LAP), N-acetyl-β-Glucosaminidase (chitin degradation; NAG), Phosphatase (P mineralization; PHOS) and β-Xylosidase (hemicellulose degradation; XYL). All the enzyme assays were set up in 96-well microplates following Bell et al. (2013). Fluorescence was measured using a microplate fluorometer (Synergy™ HTX Multi-Mode Microplate Reader, BioTek Instruments, Inc., USA). The activities were expressed as nmol h^{-1} g^{-1}dry soil.

Quantification of fungi and bacteria

DNA was extracted from 0.5 g of defrosted soil fractions using the MoBio® PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) following manufacturer instructions. The quantity and quality of extracted DNA was checked using a NanoDrop® ND-2000c UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The abundances of the bacterial (16s rRNA) and fungal (ITS) genes were analyzed using quantitative PCR (qPCR) on a CFX-96 thermocycler (Biorad, USA). Total bacterial 16S and fungal 18s rRNA genes were quantified using primer pairs Eub 338-Eub 518 and ITS1F-5.8s respectively, following Evans and Wallenstein (2012). Efficiencies for all quantification reactions were higher than 90%, with R^2 values above 0.90. The fungal: bacterial ratio was calculated using qPCR data. Results from qPCR were log-transformed for subsequent data analysis.

Statistical analyses

We first tested for significant differences in the 16 soil attributes measured across different plant and biocrust species by conducting a semiparametric MANOVA (PERMANOVA, Anderson 2001), with species as a fixed factor. Note that PERMANOVA allows unbalanced designs (i.e., different number of replicates), hence it is suitable for analyzing the data collected given our sampling design. Moreover, PERMANOVA do not require MANOVA assumptions (normality and homogeneity of variances), which were not met by our variables. We also conducted independent one-way PERMANOVA analyses for each soil and microbial variables evaluated, and tested for differences among plant and biocrust species for these variables using pairwise post hoc tests (Anderson, 2001). To help visualize the differences among species, and to aid in the interpretation of PERMANOVA results, we conducted a principal component analysis (PCA) with the 16 soil attributes analyzed. We tested for

differences in the soil variables analyzed including abundance of bacteria and fungi across different species by using one-way PERMANOVA. Before carrying out PERMANOVA and PCA analyses, the different variables measured were standardized by using the Z-score (Kreyszig, 1978). PERMANOVA analyses were developed using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymouth Marine Laboratory, Ivybridge, UK). PCA analyses were also carried out using PRIMER.

We then assessed which soil variables, among the 16 measured related to C, N, and P cycling and storage, were the most important predictors of species identity (i.e. which variables differed the most across species). To do this, we conducted a classification random forests analysis (Breiman 2001) as described in Delgado-Baquerizo et al. (2015). The accuracy importance measure was computed for each tree, and was averaged over the forest (999 trees). These analyses were conducted using the rfpermute package (Archer 2013) of the R v3.3.2 software (http://cran.r-project.org/).

Finally, we evaluated the "fertility effect" of selected species on soil attributes using the relative interaction index (RII) of Armas et al. (2004). By "fertility effect" we mean the increase or decrease of a soil attribute under a given plant/lichen/moss species regarding the value of this attribute obtained in bare ground areas. RII was calculated as (Sli – Sbg)/(Sli + Sbg), where Sli and Sbg are the values of a given soil attribute under the lichen thalli/plant canopy and in bare ground areas, respectively (Armas *et al.*, 2004). The RII index was calculated separately for each attribute and species studied, using as replicates for Sli the values obtained under each species sampled (n = 10, except for *Cladonia* sp. with n = 6), which were compared in all cases with the average of the ten replicates obtained from bare ground areas. Values of RII range from −1 to +1, with positive values indicating increases in the variable studied under the canopy of species compared to bare ground areas and negative values the opposite. To test whether RII values differed significantly from zero, we assessed their 95% bootstrap confidence interval by using the bootes R package (Kirby & Gerlanc, 2013). Differences among species in the RII values were also evaluated by using one-way PERMANOVA, with species as a fixed factor. These analyses were carried out using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package.

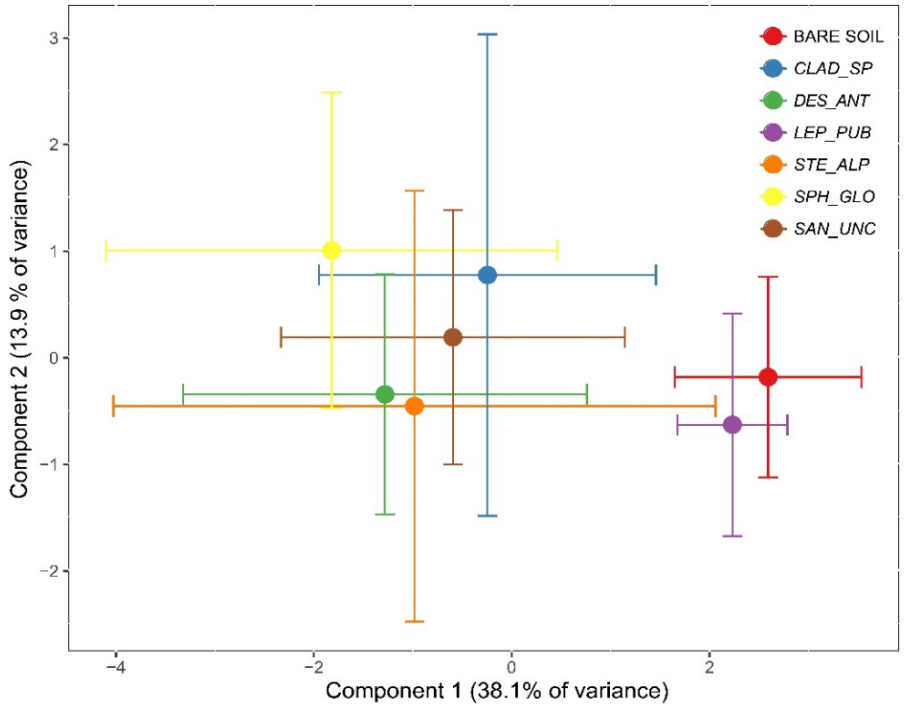
Results

Soil functioning and microbial abundance levels under different plant and biocrust species.

We found significant differences in soil functioning across different species (PERMANOVA $P < 0.001$; Pseudo-F = 3.97; d.f. = 6). In addition, we found the strongest differences in soil attribute for biocrusts vs. bare soil; and for *Leptogium puberulum* vs. all other studied species ($P < 0.001$; Table 1; Fig. 2). Similarly, *Sanionia uncinata* significantly differed to all other studied species except *Cladonia* sp. These observed differences were driven by species-associated variations of particular soil attributes and microbial abundances. However, we did not detect differences for *Cladonia* sp., *D. Antarctica*, *Stereocaulon alpinum*, and *Sphaerophorus globosus* when analyzed together (Fig. 2, Table 1).

Table 1: Results of PERMANOVA pairwise post-hoc comparisons between studied species and bare ground areas including in the analysis all the C, N, P variables evaluated. *Leptogium puberulum* (LP), *Stereocaulon alpinum* (SA), *Sphaerophorus globosus* (SG), *Cladonia sp.* (CL), *Sanionia uncinata* (SU), *Deschampsia antarctica* (DA) and bare soil (BS). *P*-values below 0.05 are in bold. (n=10, except *Cladonia* sp. with n = 6).

Species	t	*P*
LP, SA	2.1015	**<0.001**
LP, SG	2.9407	**<0.001**
LP, CL	2.0644	**<0.001**
LP, DA	2.8037	**<0.001**
LP, SU	2.4886	**<0.001**
LP, BS	1.7098	**<0.001**
SA, SG	1.0383	0.3395
SA, CL	1.0649	0.3348
SA, DA	1.0495	0.3203
SA, SU	1.4698	**0.0379**
SA, BS	2.288	**<0.001**
SG, CL	1.2458	0.1621
SG, DA	1.3281	0.1077
SG, SU	1.6947	**0.0119**
SG, BS	3.4187	**<0.001**
CL, DA	1.0963	0.3008
CL, SU	1.0294	0.4001
CL, BS	2.1376	**<0.001**
DA, SU	1.7071	**0.003**
DA, BS	3.2669	**<0.001**
SU, BS	2.9817	**<0.001**



	Component 1	Component 2
TN	-0.797 (**<0.001**)	0.361 (**0.008**)
AN	-0.498 (**<0.001**)	-0.006 (0.960)
MB-N	-0.546 (**<0.001**)	0.331 (**0.001**)
NIP	0.054 (0.669)	-0.667 (**<0.001**)
MIN	-0.751 (**<0.001**)	0.356 (**0.003**)
Corg	-0.762 (**<0.001**)	0.412 (**0.001**)
Phenols	-0.823 (**<0.001**)	0.272 (**0.027**)
Proteins	0.132 (0.292)	0.507 (**<0.001**)
DIP	-0.207 (0.095)	-0.393 (**0.001**)
AG	-0.445 (**<0.001**)	-0.356 (**0.003**)
BG	-0.928 (**<0.001**)	-0.020 (0.871)
CB	-0.877 (**<0.001**)	-0.047 (0.705)
LAP	-0.488 (**<0.001**)	0.053 (0.670)
NAG	-0.840 (**<0.001**)	-0.196 (0.116)
PHOS	-0.682 (**<0.001**)	0.045 (0.720)
XYL	-0.757 (**<0.001**)	-0.264 (**0.032**)

Figure 2: Results of a principal component analysis showing the differences between the plant and biocrust species studied according to the different soil C, N, and P functions measured. The table on the right side shows the Spearman correlations between the PCA ordination components (PC1 and PC2) and the studied soil functions (P-values < 0.05 are in bold). Values in the PCA represent means ± SD (n = 10, except for *Cladonia* sp. with n = 6). CLAD_SP: *Cladonia* sp.; DES_ANT: *Deschampsia antarctica*; LEP_PUB: *Leptogium puberulum*; STE_ALP: *Stereocaulon alpinum*; SPH_GLO: *Sphaerophorus globosus*; SAN_UNC: *Sanionia uncinata*; TN: total nitrogen; AN: available nitrogen; MB-N: microbial biomass nitrogen; NIP: potential nitrification rate; MIN: potential mineralization rate; Corg: dissolved organic C; DIP: dissolved inorganic phosphorus; AG: α-Glucosidase; BG: β-Glucosidase; CB: β-D-Cellobiosidase; LAP: L-Leucine aminopeptidase; NAG: N-acetyl-β-Glucosaminidase; PHOS: Phosphatase; XYL: β-Xylosidase.

Table 2: Soil variables measured in soils devoid of vegetation (bare soil) and in soils under six selected species. Data are means ± SE (n = 10, except for *Cladonia* sp. with n = 6). Different letters represent statistical differences among species ($P < 0.05$, PERMANOVA) for a given variable. EC: electrical conductivity (µS cm^{-1}); WHC: soil water holding capacity (%); DOC, dissolved organic carbon (g C kg^{-1}soil); Phen.: phenols (mg $C_7H_6O_3$ kg^{-1}soil); TN: total nitrogen (%); MBN, microbial biomass nitrogen (mg kg^{-1}); AN: available nitrogen (mg kg^{-1}); Prot.: proteins (mg BSA kg^{-1}soil); PMR, potential mineralization rate (mg N kg^{-1} soil day^{-1}); PNR, potential nitrification rate (mg N kg^{-1} soil day^{-1}); DIP: dissolved inorganic phosphorus (mg kg^{-1}); AG: α-Glucosidase (nmol h g^{-1}soil); BG: β-Glucosidase (nmol h g^{-1}soil); CB: β-D-Cellobiosidase (nmol h g^{-1}soil); LAP: Leucine aminopeptidase (nmol h g^{-1}soil); NAG: N-acetyl-β-Glucosaminidase (nmol h g^{-1}soil); PHOS: Phosphatase (nmol h g^{-1}soil); XYL: β-Xylosidase (nmol h g^{-1}soil).

Variable	Bare soil	*Leptogium puberulum*	*Stereocaulon alpinum*	*Sphaerophorus globosus*	*Cladonia* sp.	*Sanionia uncinata*	*Deschampsia antarctica*
pH	5.6 ± 0.09^{a}	5.5 ± 0.05^{ac}	5.3 ± 0.05^{b}	5.3 ± 0.06^{c}	5.2 ± 0.13^{b}	5.3 ± 0.10^{bc}	5.0 ± 0.13^{b}
EC	47.12 ± 6.53^{ac}	44.81 ± 2.16^{a}	71.78 ± 7.10^{bd}	$87.54\pm9,23^{b}$	64.22 ± 2.82^{cd}	37.37 ± 3.03^{a}	98.53 ± 13.53^{bd}
WHC	24.03 ± 1.16^{a}	27.65 ± 0.58^{b}	33.73 ± 1.37^{cd}	35.93 ± 1.22^{c}	31.57 ± 1.84^{cd}	30.34 ± 1.71^{bd}	33.56 ± 1.11^{cd}
DOC	11.92 ± 2.05^{ab}	7.72 ± 0.87^{a}	$23,11\pm3.15^{c}$	$27,18\pm2.35^{c}$	$19,83\pm2.54^{c}$	16.86 ± 2.45^{b}	23.11 ± 2.55^{c}
Phen.	2.68 ± 0.50^{a}	3.21 ± 0.34^{a}	7.97 ± 1.09^{bc}	9.19 ± 0.44^{b}	9.05 ± 0.91^{bc}	7.50 ± 0.67^{c}	10.78 ± 1.13^{b}
TN	0.13 ± 0.02^{ab}	0.08 ± 0.01^{a}	0.24 ± 0.04^{cd}	0.28 ± 0.02^{c}	0.26 ± 0.04^{c}	0.18 ± 0.02^{bd}	0.23 ± 0.02^{cd}
MBN	1.23 ± 0.48^{a}	3.6 ± 0.86^{b}	7.68 ± 2.17^{bc}	9.99 ± 1.93^{c}	3.44 ± 1.22^{b}	9.36 ± 2.62^{bc}	5.39 ± 1.25^{b}
AN	17.31 ± 1.10^{c}	16.99 ± 0.40^{ac}	20.03 ± 0.71^{bc}	20.12 ± 0.65^{b}	18.28 ± 1.65^{ac}	19.63 ± 0.77^{abc}	21.98 ± 0.88^{b}
Prot.	16.90 ± 2.44^{ab}	24.45 ± 4.52^{ab}	17.22 ± 4.86^{a}	24.80 ± 4.63^{b}	19.98 ± 4.48^{ab}	21.12 ± 3.92^{ab}	20.26 ± 5.41^{ab}
PMR	0.36 ± 0.12^{a}	0.93 ± 0.16^{b}	1.90 ± 0.57^{bcd}	2.73 ± 0.37^{c}	1.71 ± 0.20^{cd}	1.50 ± 0.22^{d}	2.23 ± 0.39^{cd}
PNR	0.50 ± 0.04^{ab}	0.67 ± 0.11^{a}	0.45 ± 0.09^{ab}	0.36 ± 0.08^{b}	0.55 ± 0.15^{ab}	0.49 ± 0.06^{ab}	0.66 ± 0.11^{a}
DIP	0.0213 ± 0.002^{ab}	0.0172 ± 0.001^{a}	0.0206 ± 0.001^{ab}	0.0201 ± 0.002^{ab}	0.0189 ± 0.002^{ab}	0.0186 ± 0.001^{a}	0.0222 ± 0.001^{b}
AG	23.64 ± 1.23^{a}	29.96 ± 1.09^{b}	29.15 ± 1.28^{bc}	27.85 ± 1.61^{ab}	22.40 ± 5.28^{ac}	26.78 ± 1.35^{ac}	28.19 ± 0.84^{bc}
BG	38.65 ± 3.83^{a}	34.74 ± 3.25^{a}	81.32 ± 13.71^{b}	108.28 ± 19.43^{b}	90.42 ± 22.75^{b}	89.76 ± 14.52^{b}	112.14 ± 20.00^{b}
CB	31.79 ± 2.42^{a}	23.15 ± 1.49^{b}	75.43 ± 27.61^{c}	55.94 ± 6.64^{c}	43.17 ± 8.43^{ac}	46.43 ± 5.82^{c}	49.73 ± 4.68^{c}
LAP	20.03 ± 0.73^{a}	22.14 ± 0.43^{ab}	22.25 ± 1.99^{abc}	23.62 ± 1.37^{bc}	21.62 ± 0.94^{abc}	25.34 ± 1.21^{c}	20.56 ± 0.85^{ab}
NAG	26.34 ± 1.86^{a}	30.24 ± 1.40^{a}	44.80 ± 5.93^{b}	43.13 ± 6.42^{b}	36.87 ± 6.20^{ab}	44.24 ± 4.75^{b}	42.87 ± 3.19^{b}
PHOS	342.42 ± 35.03^{ab}	311.48 ± 15.22^{a}	400.44 ± 35.25^{bc}	513.86 ± 57.16^{c}	609.74 ± 12.57^{de}	746.73 ± 49.31^{e}	563.86 ± 29.80^{d}
XYL	18.93 ± 1.14^{a}	24.30 ± 0.78^{b}	34.76 ± 4.74^{cd}	31.85 ± 3.35^{cd}	22.32 ± 6.73^{abcd}	24.91 ± 2.22^{bc}	34.97 ± 4.05^{d}

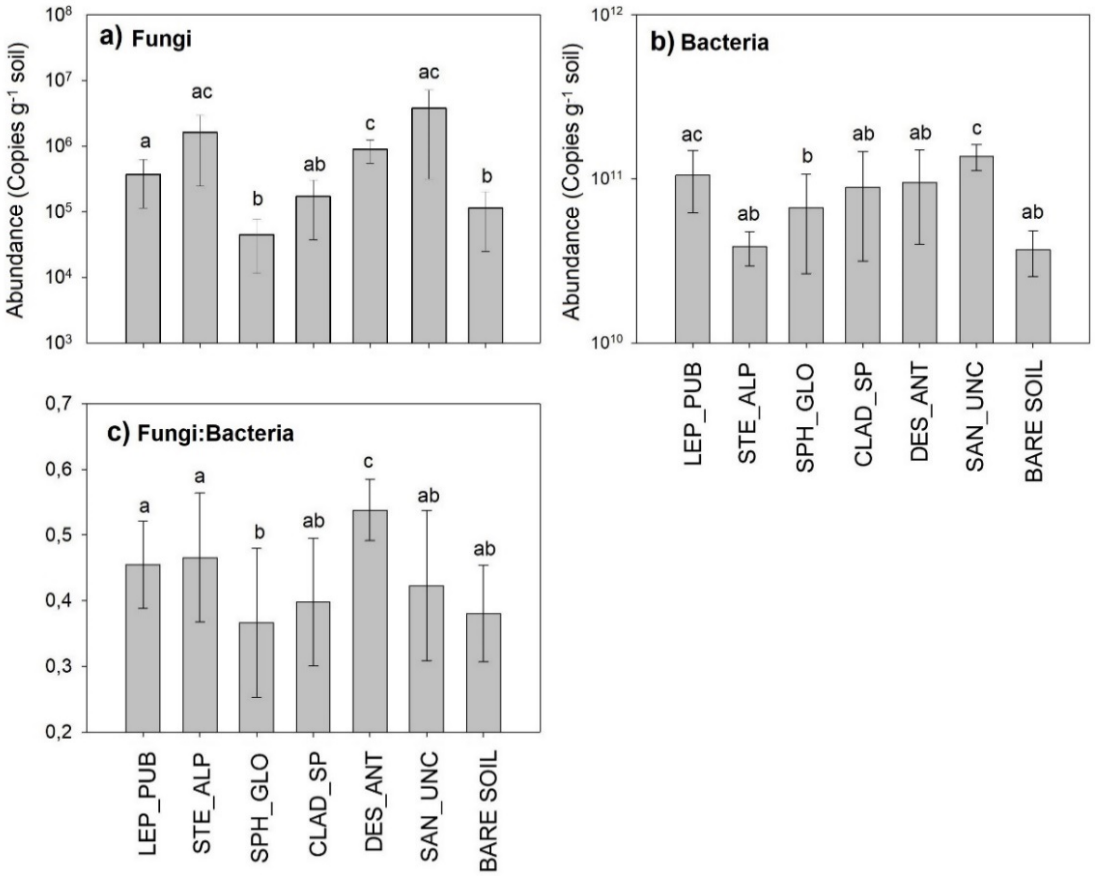


Figure 3: Abundance, quantified using qPCR, of total fungi (a) and bacteria (b) and the fungi:bacteria ratio (c). Note that different scales were employed on the y-axis of the graphs. Different letters indicate significant differences among the species studied ($P < 0.05$, post hoc test after PERMANOVA analyses). Values represent means ± SD (n = 10, except *Cladonia* sp. with n = 6). CLAD_SP: *Cladonia* sp.; DES_ANT: *Deschampsia antarctica*; LEP_PUB: *Leptogium puberulum*; STE_ALP: *Stereocaulon alpinum*; SPH_GLO: *Sphaerophorus globosus*; SAN_UNC: *Sanionia uncinata.*

The values of the soil functioning variables measured differed under each studied species. The differences between *L. puberulum* and the rest of studied species were driven mostly by the lowest values of soil nutrients (organic C, total N and phenols; $P < 0.05$; Table 2), the highest potential nitrification rate, and the values of soil enzyme activities (highest AG activity, lowest BG and CB, and lowest PHOS) found under this species ($P < 0.05$; Table 2). In the case of *S. uncinata*, its differences with the rest of species were related to the highest PHOS activity and bacterial abundance (see below), and to the intermediate values of total N and organic C found under this species (Table 2).

We found large differences in the abundance and structure (fungal:bacterial ratio) of soil bacteria and fungi across the studied species. Soils under most plant and biocrust species studied showed higher microbial abundance compared to bare ground areas, with the exception of *S. globosus* and *S. alpinum*, which had the lowest fungal and bacterial abundances, respectively (Fig. 3ab). In the case of *S. globosus*, soil fungal abundance was even lower than in soils devoid of vegetation. Soil fungal abundance under *S. alpinum, S. uncinata*, and *D. antarctica* was ~10 fold higher compared to bare ground areas. The highest soil bacterial abundances were reported under *S. uncinata* and *L. puberulum*, while soils under *S. alpinum* showed similar bacterial abundance than soils devoid of vegetation. The highest value of the fungal:bacterial ratio was observed under *D. antarctica* ($P < 0.05$, Fig. 3c). Soils under *L. puberulum* and *S. alpinum* showed intermediate values, which were different to those observed under *D. antarctica* and *S. globosus* ($P < 0.05$, Fig. 3c). *Sphaerophorus globosus* also showed similar fungal:bacterial ratio compared to soils devoid of vegetation.

Soil attributes and microbial abundance predicting observed differences across species

Our Random Forest analysis revealed that phosphatase activity, phenols, and β-D-cellobiosidase activity were the most important variables characterizing observed differences across soils under the studied species (Fig. 4), followed by other soil attributes related to nutrient availability, enzyme activities and abundance of soil microbes. However, some of the studied variables (e.g. NAG, proteins, available nitrogen, NIP, and phosphate) did not predict the differences observed across the plant and biocrust studied species.

RII index results

We observed differences in the "fertility effect" (as calculated by the RII index) of the plant, moss and lichen species studied (Fig 5). Most of the RII values obtained were positive, i.e. the concentration and rates of the analyzed soil attributes were higher under plant and biocrust species when compared to soils devoid of vegetation. The fruticose lichen *S. globosus* had the highest positive RII value for soil total N and organic C content, potential mineralization rate and microbial biomass N. *Deschampsia antarctica* showed the highest RII value for all the soil nutrients evaluated, being the only species that had positive RII value for inorganic P. Similar results were observed under the moss *S. uncinata*, except for inorganic P, which decreased under this species compared to bare ground soils. Soil nutrient content under *S. uncinata* remained at intermediate levels. However, we also detected some negative relationships between RII and particular species (e.g., *L. puberulum* and *Cladonia* sp.). *Leptogium*

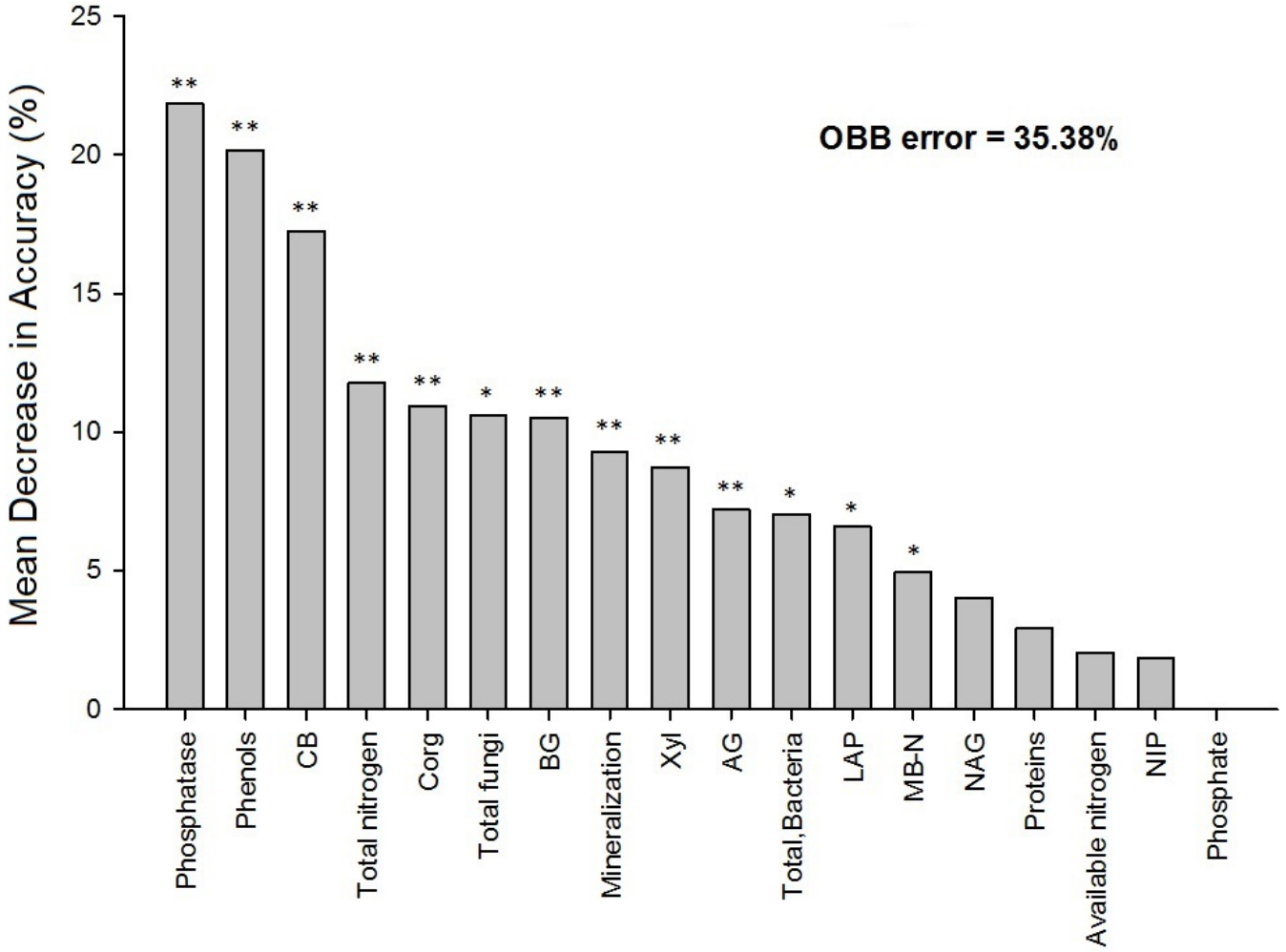


Figure 4: Random forest mean predictor importance (Mean decrease in accuracy) of soil variables studied as drivers of the observed differences in the soil variables evaluated under the canopy/thalli of the plant and biocrust species studied. Predictor importance was computed for each tree and averaged over the forest (999 trees). Significance levels are as follows: *P < 0.05 and **P < 0.01. CB: β-D-Cellobiosidase; Corg: dissolved organic C; BG: β-Glucosidase; XYL: β-Xylosidase; AG: α-Glucosidase; LAP: L-Leucine-aminopeptidase; MB-N: microbial biomass nitrogen; NAG: N-acetyl-β-Glucosaminidase; NIP: potential nitrification rate.

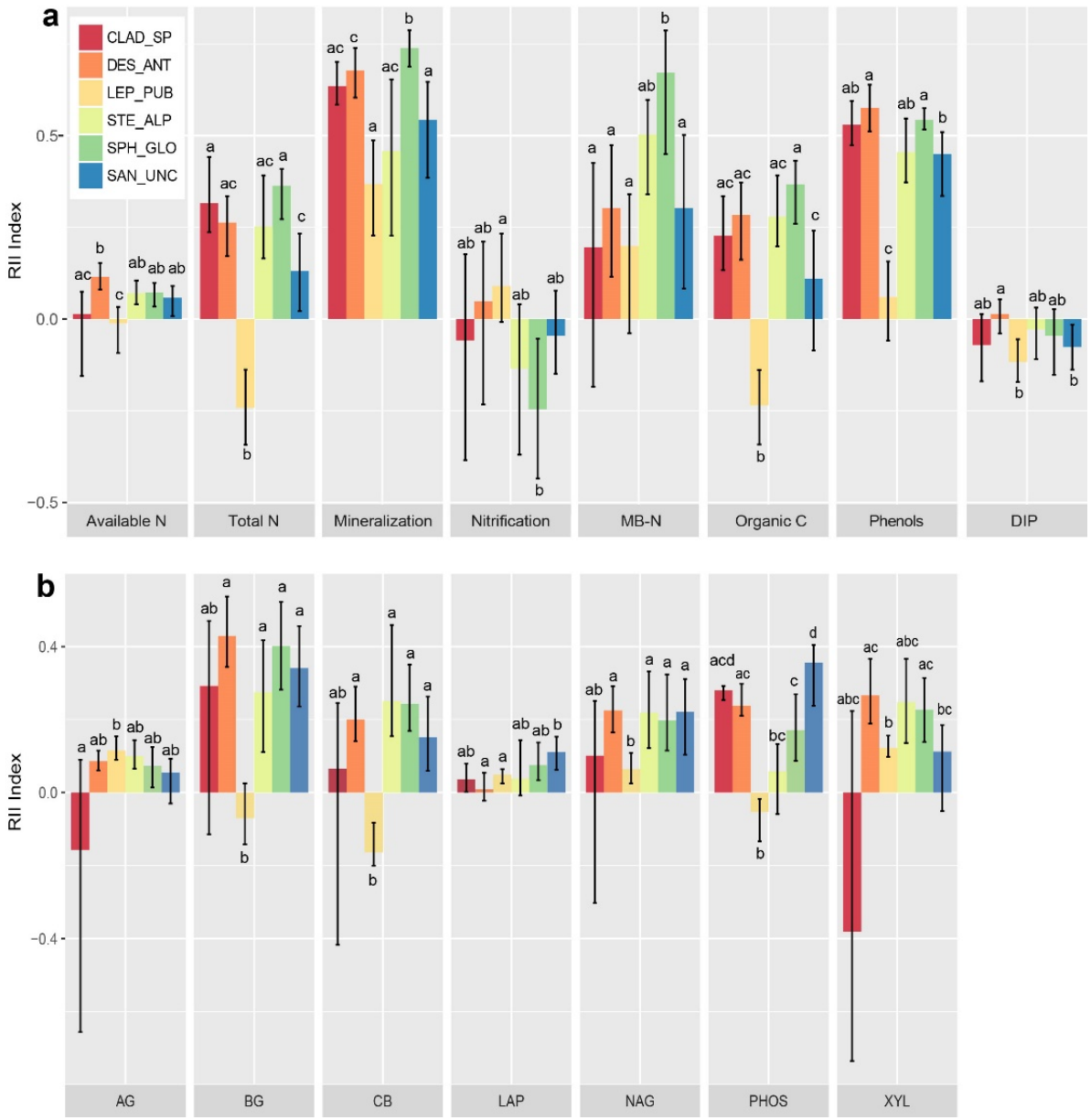


Figure 5: "Fertility effects", as measured with the relative interaction index (RII), of the species studied (vs. bare ground areas) on soil C, N and P variables (a) and soil enzymatic activities (b) evaluated. Different letters indicate significant differences between the lichen species studied ($P < 0.05$, post hoc test after PERMANOVA analyses). Values represent means ± 95% bootstrap confidence intervals (n=10, except *Cladonia* sp. with n = 6). Data for the studied soil functions that did not show significant differences between species ($P > 0.05$) are available in Fig. S1. CLAD_SP: *Cladonia* sp.; DES_ANT: *Deschampsia antarctica*; LEP_PUB: *Leptogium puberulum*; STE_ALP: *Stereocaulon alpinum*; SPH_GLO: *Sphaerophorus globosus*; SAN_UNC: *Sanionia uncinata*; N-BM: microbial biomass nitrogen; DIP: dissolved inorganic phosphorus.

puberulum showed the lowest RII values for soil total N, organic C, inorganic P, phenols and potential mineralization rates. However, this species showed the highest RII values for potential nitrification. Species-specific fertility effects were also observed when evaluating the RII values of soil enzymes (Fig 3b). *Deschampsia antarctica* and *S. uncinata* had positive RII values for soil enzymatic activities, while enzymes such as BG, CB and PHOS showed very low RII values under *L. puberulum*. Similarly, we found very low levels of RII for AG and XYL under *Cladonia* sp.

Discussion

We provide solid evidence from a comparative study that the identity of plant, moss and lichen species is associated with different levels of soil functioning and microbial abundance in maritime Antarctica. More specifically, we observed that selected plant and biocrust species were related to very different values for soil variables linked to C, N and P cycling and storage (nutrient availability and enzyme activities) and microbial abundance (total fungi, bacteria and their ratio). Our observations are supported by previous observational and experimental studies conducted in drylands (Delgado-Baquerizo *et al.*, 2015; Liu *et al.*, 2016, 2017). Among measured soil attributes, the activity of phosphatase and β-D-cellobiosidase, and the concentration of phenols, were the most important variables characterizing the differences in soil functioning across the studied plant and cryptogamic species. Different concentrations of soil phenols and surrogates of soil P cycling (phosphatase and dissolved inorganic P) have been previously reported to be associated with different biocrust species (Delgado-Baquerizo *et al.*, 2015), suggesting that these variables may consistently characterize soil-biocrust identity associations across the globe.

Soil functioning and microbial abundance levels under different plant and biocrust species

Most of the studied species were positively, but differentially, associated with soil attributes and microbial abundances when compared to bare ground areas devoid of vegetation. This positive association of vegetation patches with greater soil nutrient availability and cycling is similar to patterns previously observed in other regions (e.g. fertility islands in drylands or alpine tundra; Schlesinger et al. 1995; Cross and Schlesinger 1999; Escudero et al. 2004; Allington and Valone 2014). Here, we found that soils under particular species such as *L. puberulum* showed lower values of total and available N, organic C and inorganic P availability, which was also reduced under *Cladonia* sp. Although an explicit link with climate change is always difficult to establish using observational data, our results could

provide some insights to help predict the responses of soil functioning and microbial abundance to climate change in Antarctica. Such predictions are linked to the expected changes in the community composition of plant and biocrust communities in response to warming in this region (e.g., Amesbury et al. 2017; Lee et al. 2017; Sancho et al. 2017). For example, *D. antarctica* has experienced an expansion in some regions of the Antarctic Peninsula and associated archipelagoes (Torres-Mellado *et al.*, 2011; Cannone *et al.*, 2016). According to our results, the expansion of this species due to warmer conditions and increased growing season length, could promote an increase in the availability of N and inorganic P in Antarctic soils, positively impacting local primary productivity (Wasley *et al.*, 2006). Furthermore, it could promote an increment in soil phenolics, which may directly impact on soil microbial community composition (Qu & Wang, 2008). On the contrary, an opposite situation migth be expected with the expansion of the Antarctic endemic cyanolichen *L. puberulum*. This species predominantly occurs on temporarily wet snow beds and melt water channels (Sancho *et al.*, 1999). Thus, increased ice melt and runoff due to warming may promote its expansion, negatively influencing soil fertility (lower soil N, organic C and inorganic P concentration). However, the absence of species-specific studies dealing with species acclimation to altered climatic conditions makes it impossible to accurately predict which trend (expansion or recession) is expected for the studied species (Colesie *et al.*, 2018), which is an important topic for future research.

Soil attributes and microbial abundance drive observed differences across species

We found that phosphatase activity was the most important attribute distinguishing soil functioning across the studied species. For example, soils under *S. uncinata*, *Cladonia* sp. and *D. antarctica* had the highest phosphatase activities, while the opposite occurred under *L. puberulum* and *S. alpinum*. The capacity to obtain P is an essential functional trait for biocrust species. While C and N can be directly or indirectly obtained from the atmosphere (via collaboration with microbes; Barger et al. 2016; Sancho et al. 2016), P is mainly obtained from the bedrock (Belnap, 2011; Jones & Oburger, 2011), and therefore, the ability to obtain P will be an advantageous functional trait in these environments (i.e. bare rock left after ice retreat). Plants and biocrusts are known to influence soil P availability (Belnap *et al.*, 2003; Delgado-Baquerizo *et al.*, 2015; Mihoč *et al.*, 2016). They secrete a wide range of organic acids and powerful metal chelators, and produce phosphatases in their cell walls and mucilaginous sheaths (Jones & Wilson, 1985; Belnap, 2011). These chemical or enzymatic compounds, which are highly genus-specific in many cases, promote rock weathering and

increase the concentration of available P in the soil (Whitton *et al.*, 2005; Belnap, 2011; Jones & Oburger, 2011). Thus, differences in P acquisition traits may explain the observed differences in both P concentration and phosphatase activities in soil under selected species, reinforcing the idea that species identity has a large influence on P availability.

After phosphatase activity, the concentration of phenols was the second most important variable characterizing the observed differences in soil functioning under the studied species. Thus, we found species promoting high (e.g., *D. antarctica*) and low (e.g. *L. puberulum*) levels of phenols underneath them. We would like to highlight the case of *L. puberulum*, as soils under its thalli had low levels of soil phenols. Interestingly, this genus is known to lack typical lichen secondary metabolites (Otálora *et al.*, 2014). Similarly, the concentration of soil phenols was also a major factor charactering the differences observed among the lichen species studied by Delgado-Baquerizo et al. (2015) in a dryland ecosystem from central Spain. Phenolic substances are common UV protection compounds (Dixon & Paiva, 1995; Agati & Tattini, 2010), and are highly important for photosynthetic organisms in stratospheric ozone depleted territories such as Antarctica (Solomon, 1999, 2004). The two native Antarctic vascular plants (*D. antarctica* and *C. quitensis*) are known to synthesize and store phenolic-type molecules against UV radiation (Xiong & Day, 2001; Köhler *et al.*, 2017). For example, Ruhland et al. (2005) observed the influence of ultraviolet-B radiation on the phenylpropanoid concentrations of *D. antarctica* during the springtime ozone depletion season, observing up to 60% increase in the concentration of some phenolic substances. We observed that soils under *D. antarctica* showed the highest concentration of phenols, which may be explained by the accumulation of phenolic substances in the soil released by decaying plant material. Furthermore, phenolic compounds are also common plant root exudates with different functions (e.g. micronutrient mobility; Cesco et al. 2010), which can also act as microbial allelopathic substances. Conversely, despite its high phenolic content, soils under *D. antarctica* showed high fungal and bacterial abundances, suggesting a lack of allelopathic effects from the phenolic substances produced by this species on soil microorganisms. Similarly, phenolic derivatives are an important feature of the biochemistry of lichens, which show a great diversity of compounds that are also highly species-specific (Crittenden, 1999). Contrary to *D. antarctica*, soils under *S. globosus* thalli showed low microbial abundance (lowest fungal abundance and second lowest values for bacteria). Interestingly, soils under *S. globosus* showed the second highest concentration of phenols. This suggests that synthesized

phenolic substances by *S. globosus* may be involved, among other functions, in the chemical defense of this species against fungal activity (i.e. antimicrobial action; Lawrey 1986, 1989).

β-D-Cellobiosidase was the third most important variable characterizing the observed differences in soil functioning across the species studied. This enzyme, a cellulase, catalyzes the degradation of polysaccharides such as cellulose. This polymer is synthesized by higher plants, but also by bryophytes and, to a lesser extent, by algae and fungi (both constituents of lichen symbiosis; Haigler and Weimer 1991). Exoglucanases such as β-D-cellobiosidase are known to hydrolyze other polysaccharides (e.g. Lichenin, a storage polysaccharide found in lichens; Kanda et al. 1989; Iakiviak et al. 2011). Thus, the registered differences in soil CB activity may reflect different species-specific functional traits related to the polysaccharide content of their tissues and on their decomposability.

Microbial abundance, and fungi in particular, played a secondary but still important role in distinguishing soil functioning across species. In general, the studied species increased fungal and bacterial abundances and the fungi:bacteria ratio, in the soils under them. In addition, here we observed that plant and biocrust species patterns were related to spatial differences in soil microbial abundance. Similar results have been reported from other ecosystems (Delgado-Baquerizo *et al.*, 2016a). This is not surprising, as soil microbes are predominantly involved in soil nutrient cycling (Heritage *et al.*, 1999), and vegetation traits (e.g. SLA in plants) indirectly condition soil microbial abundance and community composition by quantity and quality of litter production (Cleveland *et al.*, 2014; Ochoa-Hueso *et al.*, 2018). Thus, differences in vegetation traits may differentially condition the observed soil microbial abundance in vegetation patches in Livingston Island. Moreover, our results match with generally reported bacterial dominance over fungi under biocrusts (Bates et al. 2010; Delgado-Baquerizo et al. 2015). The fungi:bacteria ratio was generally higher under studied species compared to bare soil, indicating an enhanced soil capacity to sequester C (Malik *et al.*, 2016). Interestingly, *S. globosus* was related to a lower fungal abundance, even lower than bare soil and differing up to two orders of magnitude with values registered under *S. uncinata*. As previously mentioned, this lower fungal abundance under *S. globosus* may reflect an antimicrobial effect of some synthetized phenolic substances (e.g. the depside sphaerophorin and the depsidone pannarin; Celenza et al. 2012, 2013).

Soil functioning under plant and biocrust species compared to bare ground areas

The values of the soil attributes evaluated were, in most cases, higher under the canopy of the studied species compared to bare ground areas. The positive relation between vegetation, including cryptogamic organisms, and soil nutrient availability compared to non-vegetated areas is largely referred to in literature (Schlesinger *et al.*, 1990, 1996; Cross & Schlesinger, 1999; Perroni-Ventura *et al.*, 2010; Concostrina-Zubiri *et al.*, 2013; Delgado-Baquerizo *et al.*, 2015). Such connection has also been reported in Antarctica for single soil attributes. For example, Beyer et al. (2000) found that soil colonization by mosses in this region coincided with higher soil organic C and N. Here we have observed similar associations, but their magnitude varied with the species and soil variable considered.

All the species except the lichen *L. puberulum* were associated with greater soil nitrogen availability. As a cyanolichen with *Nostoc* as a unique photobiont, *L. puberulum* was expected to promote higher soil N concentrations due to its N fixation capacity. Lichens (both N-fixers and non-N fixers) are susceptible to N leaching during rewetting processes (Millbank, 1978, 1982), but specifically N fixers have been proposed as important N sources in areas with low N availability (Vitousek *et al.*, 2002). Thus, the low N concentration found under *L. puberulum* may respond to biotic or abiotic factors. For instance, habitat preference (wet snow beds and melt water channels; Sancho et al. 1999) may deplete soil N availability by washing soluble compounds leached from *L. puberulum*. Conversely, lower N availability may indicate the presence of a higher rate of N transformation and cycling under this species. Supporting this idea, soils under this species showed also the highest potential nitrification rate, lowest ammonium concentration, highest nitrate concentration and highest abundance of ammonia oxidizing bacteria (unpublished data). Conversely, soils under *S. alpinum* –also a N-fixing species– did not follow the same pattern (i.e. higher values of total and available N and lower nitrification rate than *L. puberulum*). This may be a consequence of its lower N-fixation rate (compared to *L. puberulum*, data not shown) or better retention capacity of fixed N on its cephalodia (Rai 2002). Although our study was not designed to specifically assess the influence of functional traits on soil attributes, the relationship between both N-fixing lichens and soil N attributes strengthens the statement that species-specific functional traits may play an important role influencing soil biogeochemical cycles.

Phosphorus availability was mostly negatively associated with biocrust presence, as all cryptogamic species studied had lower P values in soils under their thalli (compared to bare ground areas), while the opposite occurred under the vascular plant *D. antarctica*. This may

result from a high input of organic matter deposited around this species due to sea bird nesting preferences. Some studies have reported that Antarctic sea birds (*Catharacta* spp. and *Larus dominicanus*) use *D. antarctica* communities for breeding (Albuquerque *et al.*, 2012; Parnikoza *et al.*, 2012). However, this should increase N availability as well by guano addition, and the highest levels of N were not found under this species. Although always negative, the magnitude of changes in P availability under biocrusts compared to bare ground areas differed across species. Delgado-Baquerizo et al. (2015) also observed that variables related to soil P availability showed the highest contrast among different biocrust-forming lichens in a dryland ecosystem from Spain. This again supports the idea that species identity may potentially exert a large influence on the P availability in the soil surface.

Finally, we found that the occurrence of most of the studied species was associated with greater enzyme activities under their canopy/thalli. However, differences were also observed depending on the enzyme considered. Similar species-specific associations of plant (Bell et al. (2014a) and biocrust (Liu et al. 2014) species with soil enzymes have been previously reported. Soil enzymes are fundamental drivers of organic matter degradation (Bell *et al.*, 2014b), and the presence of vegetation is considered a factor enhancing enzyme activity in comparison with bare ground areas (Gianfreda 2015). Some studies conducted in drylands (Miralles *et al.*, 2012; Zhang *et al.*, 2012; Liu *et al.*, 2014) have found that the activity of several enzymes is positively associated with biocrust-forming species (when compared to bare ground areas), a relationship that depend on the species considered. Enzyme activities are highly dependent on soil temperature and moisture (Burns *et al.*, 2013; Arnosti *et al.*, 2014; Baker & Allison, 2017), and both factors are directly associated with vegetation traits. The functional diversity of cryptogams is known to influence these soil properties by, for instance, increasing substrate temperature because of dark pigment concentration and increasing soil aggregation via the exudation of carbon compounds (Belnap *et al.*, 2003; Belnap, 2006; Almeida *et al.*, 2014). The thalli of *Sphaerophorus globosus* (unpublished data) and the lichen *Umbilicaria aprina* (Schroeter *et al.*, 2011) are known to reach temperatures above 10 ºC compared to air temperature in Antarctica. Similarly, Schlensog et al. (2013) observed differences in the temperature of thalli among Antarctic moss and lichen species. This increased temperature may enhance soil nutrient cycling under lichen thalli, naturally constrained by low soil temperature in Antarctica. Besides temperature, there are multiple other pathways for biocrust control on soil enzymes (i.e. soil pH, nutrient release or microbial activity conditioned by secondary metabolites synthesis; Hauck et al. 2009; Bowker

et al. 2011). Similarly, other microhabitat features associated with spatial patterns of species distribution may also influence enzymatic activity in soil (e.g. liquid water availability or debris accumulation by cryoturbation (Cannone *et al.*, 2008; Cannone & Guglielmin, 2010). Our data highlight the enzymes phosphatase and β-D-cellobiosidase as being greatly associated with species identity. As mentioned above, the enzyme phosphatase has previously been found highly connected to biocrust identity (Delgado-Baquerizo *et al.*, 2015) and may be a crucial variable to characterize biocrust identity effects globally. However, more efforts are needed to clarify the magnitude and pathways and explain the mechanisms by which biocrust identity regulates soil attributes such as nutrient mineralization and, consequently, to accurately predict consequences of changing species distribution for soil and ecosystem functioning in Antarctica.

Conclusions

Using a comparative approach, we provide evidence that the identity of plant, lichen and moss species was largely associated with different concentrations of soil C, N and P cycling variables and the abundance and structure (fungal:bacterial ratio) of soil microbes in maritime Antarctica. Soil phenolic content and enzymatic activity (phosphatase and β-D-cellobiosidase) were the most important variables predicting the observed differences in soil functioning across the studied species. Most evaluated species were positively associated (as measured using the RII index) to higher availability of C, N and P in soil compared to bare ground areas, which may be explained by higher soil enzymatic activities and microbial abundance. However, the magnitude of these differences was species-specific, and negative associations with some soil attributes were also observed. Our results suggest that any changes in the distribution and composition of plant and cryptogamic communities, linked to ongoing climate change or seasonal patterns, might lead to changes in the functioning and microbial abundances of Antarctic soils. They also highlight that the links between Antarctic vegetation and soil functioning are species-specific; consequently black-box approaches – considering vegetation or biocrusts as a unique entity– must be avoided to accurately characterize the role of plant and biocrust species in the functioning of Antarctic ecosystems.

Supplementary material

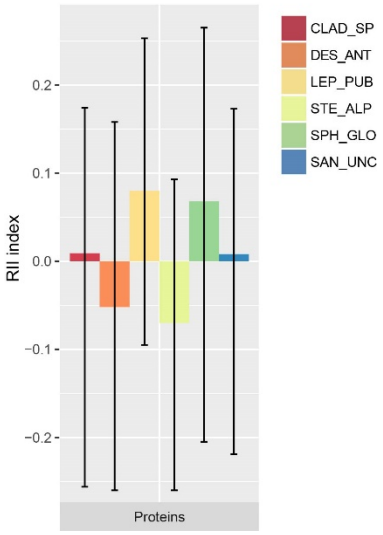


Figure S1. "Fertility effect" of the species studied (vs. bare ground areas), as measured with the relative interaction index (RII), on soil variables that did not show statistical differences between species (n=10, except *Cladonia* sp. with n = 6). CLAD_SP: *Cladonia* sp.; DES_ANT: *Deschampsia antarctica*; LEP_PUB: *Leptogium puberulum*; STE_ALP: *Stereocaulon alpinum*; SPH_GLO: *Sphaerophorus globosus*; SAN_UNC: *Sanionia uncinata*; N-BM: microbial biomass nitrogen; DIP: dissolved inorganic phosphorus.

DISCUSIÓN GENERAL

El funcionamiento de los ecosistemas de la región subantártica de Tierra del Fuego y de la Antártida marítima, y en concreto las funciones relativas a la disponibilidad y procesamiento de nutrientes, presentan una estrecha conexión con la estructura y composición de la vegetación y sus características funcionales. Esta conexión se hace especialmente relevante en el caso de la morrena del Glaciar Pía, donde la especie herbácea *Gunnera magellanica* aparece como una pieza clave en el ciclo del nitrógeno (N) en la región subantártica de Tierra del Fuego. En el **capítulo 1** se han presentado los resultados del estudio *in situ* evaluando tanto la entrada de N como su potencial destino en la cronosecuencia en un intento de explicar la extraordinariamente rápida colonización y sucesión primaria observada en esta zona. Con este estudio hemos observado que la simbiosis de plantas y microbios aparece como un elemento fundamental en estos procesos, tanto por la elevada actividad fijadora de N de *G. magellanica* como por el establecimiento de micorrizas asociadas a otras especies, lo que parece facilitar una rápida y eficiente captación del nitrógeno fijado. Debido a la naturaleza caducifolia de *G. magellanica,* gran parte de la elevada cantidad de N fijada por esta especie queda rápidamente disponible para el resto de la comunidad biótica. Sin embargo, la baja descomposición y mineralización de la materia orgánica que *a priori* podemos esperar en la zona de Tierra del Fuego, fomentan la acumulación de materia orgánica, que forma un grueso horizonte O en la zona boscosa de la cronosecuencia (Fig. 1). La simbiosis con micorrizas permitiría al resto de la vegetación, especialmente a las especies arbóreas *Nothofagus antarctica* y *N. betuloides*, competir de forma eficiente con las comunidades microbianas y captar compuestos nitrogenados directamente de la fracción orgánica del suelo, fenómeno ampliamente observado en las regiones frías de latitudes altas en el hemisferio norte (Näsholm & Persson, 2001; Näsholm *et al.*, 2008). En cuanto al fósforo (P), éste actúa como elemento limitante de la productividad primaria en ecosistemas maduros pero no suele ser un factor limitante en las primeras fases de la sucesión,

Figura 1: Potencia del horizonte orgánico (17 cm) en la zona del bosque de la morrena.

donde se considera que la presencia de P en el substrato recientemente expuesto excede los requerimiento de la comunidad biótica (Walker & Syers, 1976; Menge *et al.*, 2012). Además, suponiendo una baja disponibilidad de P, la abundancia de N permitiría a la vegetación invertir energía en sintetizar fosfatasas que mineralizan P y permiten incrementar la captación de P proveniente de la materia orgánica del suelo (Treseder & Vitousek, 2001; Houlton *et al.*, 2008). Así, la rapidez de esta sucesión primaria podría deberse a su singularidad respecto a un eficiente acceso y captación de nutrientes, donde la elevada fijación de N suprime la limitación de N típica de estos sistemas (Chapin *et al.*, 1994; Castle *et al.*, 2017). Por lo tanto, la productividad primaria en la morrena del Glaciar Pía no se encontraría limitada por la disponibilidad de N, tal y como demuestra los valores observados de N:P en tejidos de diferentes especies vegetales, y las tasas de crecimiento estimadas para las especies arbóreas se encuentran próximas al límite superior del rango habitualmente observado en especies del género *Nothofagus*. Esto, sumado a la abundante precipitación en la morrena del glaciar, parecen indicar que las condiciones son óptimas para desarrollo de la vegetación, a pesar de que la temperatura media del mes más cálido apenas alcanza los 10 ºC (Santana *et al.*, 2006).

En cuanto al papel de la vegetación criptógama, nuestros resultados son congruentes con trabajos previos analizando en laboratorio las tasas de fijación de especies de musgos (Arróniz-Crespo *et al.*, 2014) y líquenes (Raggio *et al.*, 2012) de la misma cronosecuencia. Sin embargo, las especies analizadas aquí son sólo una mínima parte de la diversidad criptogámica que aparece en diferentes fases de la sucesión, muchas de ellas simbiontes con cianobacterias (Fig. 2). Por ejemplo, el liquen *Placopsis pycnotheca* (Fig. 2a) es una especie extremadamente abundante en las fases iniciales donde forma grandes extensiones de costra biológica (De los Ríos et al. 2011). Esta especie presenta mayores tasas de fijación de N que la especie del mismo género aquí analizada (Raggio *et al.*, 2012). Además, muchos grupos no han sido evaluados aquí, como la planta *Anthoceros* (Fig. 2b), muy común en los bosques maduros de Tierra del Fuego, que también establece simbiosis con cianobacterias y aporta N al medio (Rai *et al.*, 2002). Por ello, es posible considerar que la entrada de N por medio de la actividad diazótrofa en criptógamas y otros organismos es también considerable, destacando aún más la posible singularidad de esta región.

En el **capítulo 2** se muestra la variabilidad morfológica y fisiológica de *G. magellanica* a lo largo de un gradiente altitudinal que pretende recoger la amplitud del nicho ecológico de esta especie en Tierra del Fuego, pues incluye poblaciones a nivel del mar y también en el

límite altitudinal del bosque y en la tundra alto-andina en Isla Navarino. El hecho de que la tasa de fijación de N sea prácticamente la misma a nivel del mar que en la tundra (todo ello debido a lo que parece un cambio de estrategia en el crecimiento de la planta pasando de invertir energía en estructuras de crecimiento y fotosíntesis en las zonas bajas a invertir en estructuras de reproducción y supervivencia en la zona de tundra) sugiere que la relevancia de esta especie no sólo se restringe a suelos recientemente expuestos tras el retroceso glaciar sino también a ecosistemas maduros y con gran estrés ambiental, aunque en este último caso la cobertura de esta especie sea menor. Así, la presencia de *G. magellanica* se traduce en un aporte considerable de N al ecosistema, lo cual confirma que la alta fijación de N observada es algo ampliamente extendido por esta región asociado a la presencia de *G. magellanica*. Todo esto apunta a que Tierra del Fuego podría considerarse como una de las zonas con mayor fijación de N a nivel global. Además, considerando el área de distribución de *G. magellanica* y las demás especies del género *Gunnera*, obviadas por los estudios locales y las estimaciones globales de fijación de N, la verdadera relevancia de esta región en el ciclo del N a nivel global parece estar infravalorada.

Los resultados obtenidos sobre el potencial papel de *G. magellanica* como motor de una sucesión vegetal extraordinariamente rápida y la robustez en sus tasas de FBN tanto a nivel del mar como en la tundra alto-andina incitan a especular sobre cuál podría ser el impacto de esta especie si llegase a colonizar otros territorios como, por ejemplo, la Antártida marítima. Si bien es una situación poco probable a corto plazo, pues ya se demostró que *G. magellanica* no es capaz de sobrevivir al invierno antártico en las Islas Orcadas del Sur (Edwards & Greene, 1973; Smith, 1996), el actual aumento de temperatura en esta zona

Figure 2: Diferentes organismos simbiontes con cianobacterias que intervienen en la sucesión primaria en Tierra del Fuego. (a) Comunidad de costra biológica incluyendo el liquen *Placopsis pycnotheca*; (b) *Anthoceros* sp.; (c) cianolíquenes *Sticta hypochra* y *Psoroma hypnorum.*

podría favorecer esta posibilidad. De hecho, las especies *Gamochaeta nivalis* y *Nassauvia magellanica*, especies habituales de la flora orófila propia de la tundra subantártica en Tierra del Fuego junto con *G. magellanica*, han sido recientemente localizadas en Isla Decepción (Islas Shetland del Sur), y se observó que habían sobrevivido durante varios años e incluso podrían haberse reproducido asexualmente (Smith & Richardson, 2010). Por ello, dado el solapamiento en las áreas de distribución y nicho ecológico, convendría evaluar la capacidad invasiva de la especie *G. magellanica*, determinando su potencial impacto para el funcionamiento de los ecosistemas de la Antártida marítima y demás regiones subantárticas.

En el **capítulo 3** se muestran los cambios altitudinales de la diversidad y composición de las comunidades microbianas en Isla Navarino, explorando los factores ambientales (tanto bióticos como abióticos) que explican dichos cambios. En general, observamos que la transición entre el bosque subantártico magallánico y la tundra alto-andina (o tundra subantártica) desencadenó profundos cambios tanto en la estructura como en la composición de la comunidad microbiana. De hecho, atributos relacionados con la vegetación, como son la diversidad de plantas vasculares, la productividad primaria y el cambio de hábitat (bosque-tundra) fueron los predictores más importantes de la diversidad y abundancia de microorganismos. Sin embargo, la respuesta de los grupos taxonómicos y funcionales a la altitud fue muy dependiente del taxón considerado. De igual manera, la relación de los grupos de microbios con las diferentes funciones del suelo fue muy específica para cada grupo. Estos resultados remarcan la estrecha conexión entre los diferentes componentes de la comunidad biótica y en concreto, entre la vegetación y la microbiota del suelo. Sorprendentemente, no observamos que el pH fuera un factor importante para los microorganismos del suelo, pese a que distintos estudios lo han destacado como un determinante clave de las comunidades microbianas (Fierer & Jackson, 2006; Shen *et al.*, 2013; Delgado-Baquerizo *et al.*, 2018a). No obstante, el pH no siguió ningún patrón concreto con la altitud en nuestro gradiente. De igual manera, la ausencia de conexión entre las actividades enzimáticas analizadas y la abundancia y diversidad (taxonómica y funcional) de grupos microbianos y las escasas asociaciones observadas entre estos y la disponibilidad de nutrientes fue inesperada.

En el **capítulo 4** se muestra la importancia de la vegetación y la costra biológica del suelo para el funcionamiento de los ecosistemas antárticos. En concreto, nuestros resultados muestran que la presencia de vegetación se asocia en general con una mayor disponibilidad de nutrientes y abundancia de microorganismos. Estos resultados sugieren que la teoría de las islas de fertilidad (Schlesinger *et al.*, 1996), inicialmente propuesta para ecosistemas áridos donde la vegetación tiende a agruparse formando manchas discretas rodeadas por una matriz de suelo sin vegetación vascular perenne (Maestre & Cortina, 2002; Kéfi *et al.*, 2007), puede aplicarse también a los ecosistemas antárticos en donde la vegetación no forma un continuo sino que aparece relegada a zonas dispersas (en este caso por fenómenos distintos de la aridez). Así, los suelos desprovistos de vegetación presentarían menor contenido en nutrientes, menor abundancia de microorganismos y menor actividad enzimática. Sin embargo, la presencia de vegetación no se asocia de manera homogénea a unos mayores niveles de funcionalidad. Hemos podido observar que esta relación varía en función de la especie que recubre el suelo, existiendo conexiones especie-específicas que hacen que varias

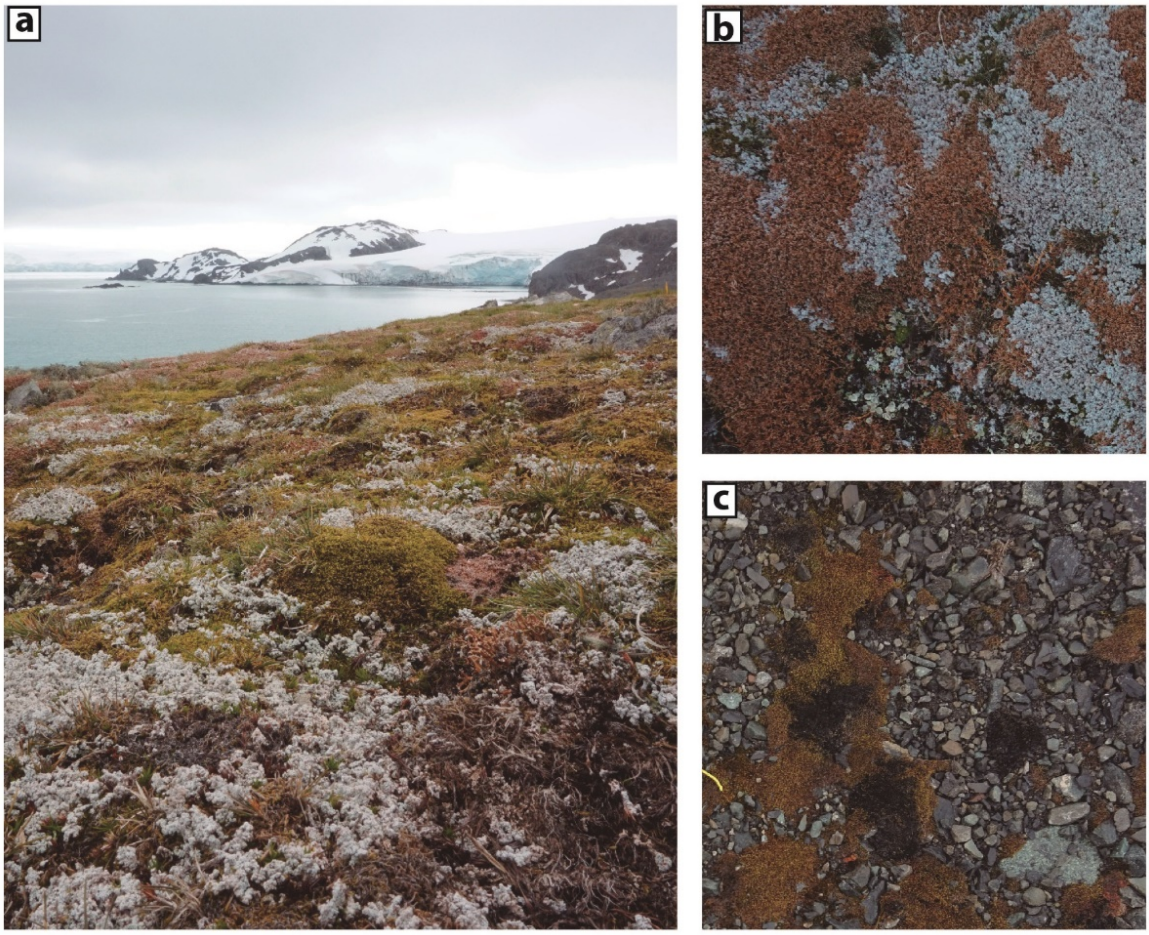


Figure 3: (a) Comunidad formada fundamentalmente por especies criptógamas; (b) parches monoespecíficos del musgo *Sanionia uncinata* y el liquen (negro) *Leptogium puberulum*; (c) talos del liquen *Stereocaulon alpinum* sobre sustrato rocoso.

especies que formen parches monoespecíficos contiguos (Fig. 3) se relacionen con niveles diferentes de funciones concretas. Estos resultados tienen gran interés a la hora de conocer el funcionamiento de estos ecosistemas. Además, nuestros resultados apuntan a una cuestión que no hemos abordado directamente pero que durante los últimos años está siendo ampliamente estudiada en el caso de las comunidades de criptógamas: la diversidad funcional y sus relevancia ecológica (Cornelissen *et al.*, 2007). Estudiar la diversidad funcional de estas especies en la Antártida, el único continente dominado por vegetación criptogámica, es necesario si deseamos comprender en profundidad su papel en los ciclos biogeoquímicos de esta región. Las diferencias especie-específicas observadas en el **capítulo 4** pueden estar relacionadas con rasgos funcionales de las especies estudiadas no considerados en nuestro trabajo, como por ejemplo sugieren los diferentes valores de N observados bajo dos especies simbiontes con cianobacterias: *Leptogium puberulum* y *Stereocaulon alpinum* (Fig. 3). Tanto el biotipo (escuamuloso y fruticuloso, respectivamente) como el tipo de simbiosis (*Nostoc* como simbionte primario y secundario, respectivamente) pueden estar determinando diferencias en las tasas de fijación o la capacidad de retención del nitrógeno fijado, y por lo tanto, en las funciones relacionadas con el ciclo del N bajo ambas especies. Lo mismo puede suceder con diferencias en los tiempos de retención de agua en el talo, la capacidad de captar partículas transportadas por el viento, etc. (Mallen-Cooper & Eldridge, 2016). Además, sería necesario conocer más sobre los procesos de colonización de las especies antárticas, así como los factores a nivel de micro-hábitat que condicionen su establecimiento en determinadas regiones. Debido a que múltiples especies antárticas están ya viendo alterados sus patrones de distribución, es urgente ampliar nuestro conocimiento sobre la importancia de la identidad taxonómica en el funcionamiento de estos ecosistemas, poniendo especial énfasis en comprender e identificar los mecanismos por los cuales dicha identidad se asocia con determinados niveles de nutrientes, actividad enzimática o demás funciones del suelo. Así podríamos predecir de forma más precisa cómo pueden verse afectados tanto las especies como los ecosistemas antárticos en respuesta al cambio climático en que nos encontramos inmersos.

CONCLUSIONES

1. La especie *Gunnera magellanica* Lam. se ha revelado como un elemento crucial para el funcionamiento de los ecosistemas fueguinos, presentando la tasa de fijación de nitrógeno (N) más alta observada entre las plantas vasculares que forman simbiosis con cianobacterias, similar a las tasas más altas registradas hasta la fecha con plantas vasculares (leguminosas, plantas actinorrízicas como *Alnus* spp. o el helecho acuático *Azolla* spp).

2. La extraordinariamente rápida sucesión primaria observada en Tierra del Fuego (Glaciar Pía) podría deberse a la elevada entrada de N proveniente de la actividad diazótrofa de la especie *Gunnera magellanica*, junto con la eficiente captación y transferencia de N entre la vegetación por medio del establecimiento de micorrizas.

3. Las tasas de fijación de N obtenidas para las especies estudiadas de líquenes son también elevadas, siendo en el caso de *Peltigera patagonica* y *Placopsis perrugosa* equiparables en peso seco a la tasa obtenida para la especie *G. magellanica,* pero su presencia restringida a fases muy iniciales de la sucesión limitan su relevancia como fuente de N en los ecosistemas de Tierra del Fuego.

4. La especie *G. magellanica* mostró marcadas adaptaciones morfológicas a las condiciones climáticas desfavorables propias de la tundra alto-andina, pero dichas adaptaciones no se observaron a nivel fisiológico o funcional. Su relevancia como fuente de N se mantuvo constante debido a un cambio en la estrategia de crecimiento de la especie.

5. La diversidad y composición de la comunidad microbiana se vio fuertemente afectada a lo largo del gradiente altitudinal estudiado en Isla Navarino, siendo los atributos de la vegetación (riqueza de especies, productividad primaria neta y cambio de hábitat) los más importantes predictores de dichos cambios. No obstante, la respuesta de la diversidad microbiana fue muy variable en cuanto al grupo taxonómico estudiado, mientras que la abundancia de los grupos taxonómicos y funcionales analizados siguió un patrón común.

6. La abrupta transición entre el bosque subantártico magallánico y la tundra alto-andina en Isla Navarino desencadenó cambios importantes en la diversidad y composición de las comunidades microbianas, siendo muy evidentes en la comunidad fúngica, donde se pasó de una comunidad dominada por especies micorrízicas a una comunidad dominada por especies endófitas.

7. Los patrones altitudinales de grupos taxonómicos y funcionales claves en la comunidades microbianas a lo largo del gradiente analizado coincidieron con los patrones observados para

diferentes atributos del suelo a lo largo del gradiente analizado, por lo que cabe prever que cambios en la comunidad microbiana o en funciones concretas del suelo como consecuencia de alteraciones en las condiciones climáticas en la zona repercutirán de forma recíproca en el funcionamiento de los ecosistemas fueguinos.

8. La presencia de vegetación en la Antártida marítima está asociada positivamente a una mayor disponibilidad de nutrientes en el suelo, sugiriendo que la teoría de las islas de fertilidad es también aplicable a los ecosistemas antárticos.

9. La relación de las especies vegetales antárticas con las funciones del suelo analizadas resultó ser diferente en función de la especie considerada, lo cual indica que cambios en la composición y abundancia de especies vegetales como consecuencia de los efectos del actual cambio climático pueden conllevar cambios importantes en el funcionamiento de los ecosistemas terrestres antárticos.

10. Las funciones del suelo que más dependieron de la identidad taxonómica de la vegetación en Isla Livingston fueron el contenido en sustancias fenólicas y la actividad de las enzimas fosfatasa y β-D-celobiosidasa, siendo quizá las variables que mejor caracterizan los efectos de la vegetación criptogámica sobre el funcionamiento del suelo a nivel global.

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